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Flame Photometry Speeds Infant Blood Studies

Flame photometer used in daily blood ultra-micro analyses on infants at Rockford Memorial Hospital.

Preliminary results of a study on premature and normal-term (but immature) infants indicate that high mortality in the group is due, in part, to the high incidence of adrenal immaturity. The study is being made at Illinois' Rockford Memorial Hospital, with the cooperation of the local Board of Health, and other hospitals. Special ultra-micro blood analysis techniques have been developed by Dr. Samuel Natelson, Chairman of Rockford Hospital's Department of Biochemistry.

Daily Microanalyses—In order to maintain these infants in physiological balance, daily analyses of the blood are made in the first weeks of life. To allow such analyses, ultra-micro procedures have been made routine for the estimation of Na, K, Cl, protein, hematocrit, non-protein nitrogen, sugar, and urea. All of these determinations are made on one sample (approx. 0.1 ml) of capillary blood drawn from the punctured heel or fingertip. Some of the subjects have been followed for more than a year during this study.

Procedure—A Perkin-Elmer Model 52-A Flame Photometer is used for the Na and K determinations. An aliquot of plasma solution containing 0.02 ml of plasma is mixed with 4 ml of lithium solution containing 0.5 mg per ml of solution as lithium. Dr. Natelson employs a slow-flow atomizer, in which he has readjusted the position of the jets to spray about 2 ml in 18 to 20 seconds at an air pressure of 10 pounds. Only 2 ml of final solution are used for each Na or K measurement. Complete details on standard solutions, as well as on reagents and construction of pipets for the other ultra-micro procedures can be found in Dr. Natelson's paper, *Am. J. Clin. Path., 21,* 12, 1951.

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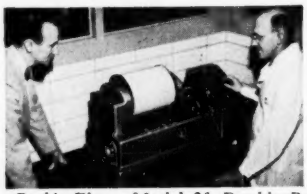

The Perkin-Elmer Model 21 Double Beam Spectrophotometer at Smith, Kline & French

Assaying chemically similar compounds is an oft-encountered problem in every chemical and research laboratory. Smith, Kline & French Laboratories applied infrared methods of analysis to the assay of khellin and visnagin, two similar new drugs from crude seed extracts of *Ammi visnaga,* Lam. Now quantitative and qualitative assays of these drugs can be made in only 20 minutes on plant fractions and pharmaceutical mixtures. A Perkin-Elmer Model 21 Spectrophotometer with NaCl optics is used, with a standard liquid cell. The same instrument is also used for differentiating between chemically similar drugs and new product development.

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REMOVAL OF CALCIUM IN MAN BY ETHYLENEDIAMINE TETRA-ACETIC ACID. A METABOLIC STUDY *

By HERTA SPENCER, VERNICE VANKINSCOTT, ISAAC LEWIN,[1] AND DANIEL LASZLO

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(Submitted for publication July 7, 1952; accepted September 5, 1952)

Ethylenediamine tetra-acetic acid (EDTAA) forms soluble complexes with divalent and trivalent metallic ions (1). This chelation was found to be preferential and depends on the pH of the solution. The binding power of this agent for metals is so great that once the metal is chelated it cannot be precipitated by the common precipitating agents.

Calcium was shown to form a complex with EDTAA at a pH above 6.5. With increasing hydrogen ion concentration, dissociation occurs and is complete at pH 5. The ability of EDTAA to form complexes with calcium, magnesium, lead and other metals has been studied *in vitro* and *in vivo* (2a, b; 3a, b, c, d; 4a, b,). Rapid intravenous injection of the di-sodium salt of EDTAA (di-Na-EDTA) into rabbits lowered the serum calcium precipitously to levels causing tetany; these changes could be reversed by the intravenous administration of calcium salts. On the other hand, slow intravenous injection of large quantities of this agent caused no significant decrease of the serum calcium nor any untoward effects (2a). The pharmacological properties of this agent were studied in animals (3c), and its effects upon coagulation were investigated (2b).

This agent was investigated in man for the applicability of the above results obtained experimentally. An agent capable of disturbing calcium homeostasis by altering the serum calcium level would be a useful tool of investigation of mineral metabolism. It may also be of therapeutic value in states of deranged calcium metabolism. A comparison of the metabolic effects of the sodium salt with that of the calcium salt of EDTAA (di-Na-Ca-EDTA) appeared of interest. The latter does not interfere with calcium homeostasis and yet can exchange its calcium with endogenous ionized calcium or with other metals deposited in bone (3a, b, c, d; 4a; 5). Therefore, the effects of the sodium and calcium salts of this compound upon calcium metabolism were studied under controlled conditions in 10 and 5 patients, respectively (Table I). Four of these patients had no bone disease (Cases 2, 3, 8, 10), two had osteoporosis (Cases 1, 4), two had osteoblastic metastases secondary to prostatic carcinoma (Cases 7, 11), two had osteolytic metastases secondary to carcinoma of the breast (Cases 5, 9) and one had multiple myeloma (Case 6).

METHODS

The patients were observed on the metabolic ward and received a low calcium diet (6). The study was divided into three phases: *1)* a control period of at least six days; *2)* an experimental period of six days during which one to six infusions were administered; *3)* a post-infusion period of at least six days. The food, urine, and stools were analyzed for nitrogen, phosphorus, sodium and potassium as previously described (7a, b). The calcium content of the food was analyzed in the usual way (8). After ashing of the acidified urine and stool in the muffle furnace, the urinary and fecal calcium were determined. The urine was analyzed for calcium daily, and in some instances, 24-hour urine collections were fractionated into six hour specimens. The stool analysis for calcium was performed on samples of a six-day pool. The serum calcium was determined simultaneously by direct oxalate precipitation as well as by oxalate precipitation of the acid ashed sample.

A twenty per cent solution of di-Na-EDTA was prepared and adjusted to a pH of 7.4 by addition of sodium hydroxide. Di-Na-Ca-EDTA was obtained as a 20% solution in ampules.[2] A solution containing 4 grams of either of these salts was added to 500 cc. of 5 per cent glucose in water and was infused over a period of six hours on one to six consecutive days. Some patients received *2* grams of the sodium salt or of the calcium salt as the first dose, and two patients received *2* grams of

* This study was supported in part by a Research Grant (C-1540 [C]) from The National Cancer Institute of The National Institutes of Health, Public Health Service.

[1] Trainee, National Cancer Institute.

[2] The sodium and calcium salts of EDTAA were kindly supplied by the Bersworth Chemical Company, Framingham, Mass., and by Rikers Laboratories, Inc., Los Angeles, California. The authors thank Dr. John J. Singer and Dr. George L. Maison, for their kind cooperation.

the sodium salt daily for six days (Table I). The patients were observed during the entire period of infusion; temperature, pulse, respiration and blood pressure were determined frequently. Serum calcium was determined before and at the end of the infusion, and in some instances also at frequent intervals during the infusion.

RESULTS

Metabolic observations.—Table I shows the effects of intravenously administered di-Na-Ca-EDTA, of di-Na-EDTA, and of calcium gluconate upon the 24-hour urinary calcium excretion. In four cases (Cases 2, 3, 4, 11) the comparative effects of all three substances were studied in the same patient. The calcium salt of the complexing agent was administered first, and after its effects subsided, the sodium salt was given.

Excess urinary calcium excretion was observed in every instance on the day of infusion. This excess was calculated by subtracting the 24-hour urinary calcium excretion prior to infusion from the

TABLE I

Effect of sodium salts and calcium salts of ethylenediamine tetra-acetic acid and of calcium gluconate upon urinary calcium excretion

Case	Infusate*	24-Hour urinary calcium excretion in mg.										
		Prior to infusion†	During infusion									After infusion
			Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Average	Excess‡	Theoretic§ excretion	
										mg.	*%*	
1	di-Na-EDTA	41	169*	304	251	455	309	331	287	246	62	55
2a	di-Na-Ca-EDTA	69	408	389	384	—	—	—	394	325	82	61
b	di-Na-EDTA	75	288	356	320	—	—	—	321	246	57	61
c	Calcium gluconate	70	286	—	—	—	—	—	286	216	48	75
3a	di-Na-Ca-EDTA	108	286*	363	385	—	—	—	365	257	79	118
b	di-Na-EDTA	89	208*	373	361	—	—	—	314	225	63	107
c	Calcium gluconate	103	256	—	—	—	—	—	256	153	34	121
4a	di-Na-Ca-EDTA	20	286	361	354	—	—	—	367	347	88	35
b	di-Na-EDTA	26	346	344	310	—	—	—	333	307	71	40
c	Calcium gluconate	27	159	—	—	—	—	—	159	132	30	43
5	di-Na-EDTA	14	324	329	299	—	—	—	317	303	70	10
6a	di-Na-EDTA	54	432	335	321	—	—	—	363	309	71	30
b	Calcium gluconate	60	172	—	—	—	—	—	172	112	25	65
7a	di-Na-EDTA	20	255	279	236	—	—	—	257	237	55	9
b	Calcium gluconate	68	216	—	—	—	—	—	216	148	23	85
8	di-Na-Ca-EDTA	89	334	—	—	—	—	—	334	245	63	175
9a	di-Na-EDTA	43	186*	194*	174*	197*	185*	172*	185	142	66	21
b	Calcium gluconate	34	186	—	—	—	—	—	186	152	34	43
10	di-Na-EDTA	85	215*	192*	188*	182*	130*	182*	181	96	45	69
11a	di-Na-Ca-EDTA	18	313	—	—	—	—	—	313	295	75	30
b	di-Na-EDTA	32	343	—	—	—	—	—	343	311	72	84
c	Calcium gluconate	43	75	—	—	—	—	—	75	32	7	50

* Each infusion contained 4 gm. of the sodium or calcium salt. In the cases indicated by asterisks only 2 gm. were infused. Calcium gluconate infusions contained 50 ml. of a 10% solution or 446 mg. of calcium.

‡ Excess = Average 24-hour urinary calcium excretion during the infusion minus the pre-infusion level.

§ Per cent of theoretic excretion = $\frac{\text{Excess Excretion}}{\text{Theoretic Excretion}} \times 100$. Theoretic excretion: 1 gm. di-Na-EDTA complexes with 108 mg. calcium; 1 gm. di-Na-Ca-EDTA contains 98 mg. calcium.

† The average 24-hour urinary calcium excretion of a large number of patients without bone disease observed on the metabolic ward while on a low calcium diet (150 mg. calcium/day) is 71 ± 9 mg. Cases 2, 3, 8, 10, were normals. Cases 7 and 11 had osteoblastic metastases secondary to carcinoma of the prostate with a lower than normal calcinuria characteristic for this condition (7a). Cases 4, 5 and 9 received hormonal therapy for a prolonged period of time.

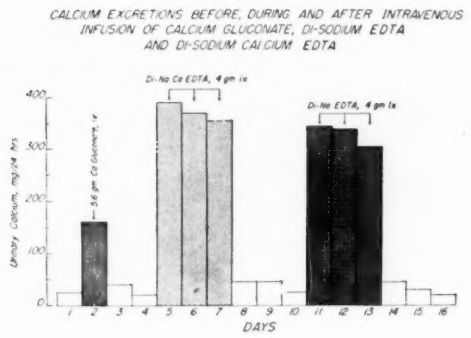
CALCIUM EXCRETIONS BEFORE, DURING AND AFTER INTRAVENOUS INFUSION OF CALCIUM GLUCONATE, DI-SODIUM EDTA AND DI-SODIUM CALCIUM EDTA
Di-Na Ca EDTA, 4 gm iv
Di-Na EDTA, 4 gm iv
3.6 gm Ca Gluconate, iv
Urinary Calcium, mg/24 hrs
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
DAYS

FIG. 1

average 24-hour urinary calcium excretion on the day of the infusion. The excess following the infusion of the sodium salt expressed in per cent of the expected calcium binding power of this agent,[3] ranged from 45 to 72 per cent. The surplus urinary calcium excreted following the infusion of the calcium salt was somewhat higher and ranged from 63 to 88 per cent of the injected dose. The excess urinary calcium excretion following the infusion of calcium gluconate was, in every instance, significantly lower than after the administration of the salts of EDTAA (Table I and Figure 1).

The rate of urinary calcium excretion measured in six-hour intervals on the day before, during and after the infusion of di-Na-EDTA is shown in Figure 2. The first six-hour urine collection coincided with the time of the infusion. Twenty-eight per cent of the excess calcium was excreted *during* the infusion, whereas 60 per cent was excreted in the six-hour period *following* the infusion. A significant excess of urinary calcium was still present in the subsequent 12 hours. When the calcium salt was administered, the maximum urinary calcium excretion was noted *during* the time of infusion.

The urinary calcium excretion on the day after the infusion usually approximated the pre-infusion level. Only in one out of five patients injected with the calcium salt was the calcium excretion on the day after the infusion significantly higher than on the day preceding the infusion (Case 8).

The calcium balances reflected the increased urinary calcium excretion; there was no significant change of the fecal calcium content. The serum calcium levels determined by direct oxalate precipitation showed no significant lowering during or after the infusion of the sodium salt. However, the total calcium determined on the acid ashed serum was significantly higher during the infusion and up to 18 hours after its completion. The highest increment representing the EDTA-bound calcium, non-precipitable by oxalate, was 4.3 mg. %.

Changes of urinary phosphorus excretion and of the phosphorus balance were less constant; only in two cases (Cases 1, 3) was there a marked increase in phosphaturia and a tendency toward decreased positivity of the phosphorus balance. The nitrogen and sodium balances remained unchanged. In two cases (Cases 1, 4) the urinary potassium excretion was increased. In all cases studied, the levels of the serum phosphorus, sodium, potassium, and alkaline phosphatase remained unchanged.

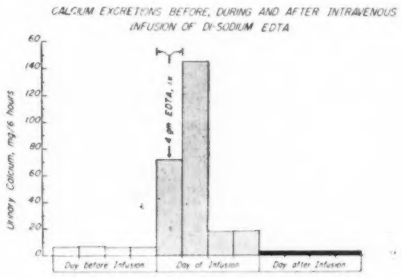

FIG. 2

Clinical observations.—No significant changes of pulse rate, temperature, or of respiratory rate were noted during the infusion. Blood counts were followed for several weeks after the administration of the salts of EDTAA and they showed no changes which might have been attributed to these agents. The urine and the blood urea nitrogen showed no abnormalities. The systolic blood pressure was significantly lowered in five out of nine cases checked during the infusion of the sodium salt (11 times out of 18 infusions) and in four out of five cases receiving the calcium salt (six times out of ten infusions), with a maximal depression of 30 mm. Hg (9). The diastolic pressure was only occasionally lowered (maximal depression 20 mm. Hg). Although the fall of the blood pressure was

[3] 1 gm. di-Na-EDTA complexes with 108 mg. calcium.

greatest in the second and third hour of the infusion, it occurred in the first hour in one case (Case 9). It rose usually to pre-infusion levels in one to two hours after the completion of the infusion.

DISCUSSION

The present study has shown that the slow infusion of di-Na-EDTA causes no lowering of the serum calcium levels in man. This confirms earlier observations in animals (2). The maintenance of serum calcium levels, while infusing a calcium binding agent, and the concomitant excess calcinuria suggest that any decrease of the ionized serum calcium is corrected by prompt replenishment from the large calcium depots of the body, namely, from the skeletal system. This agent, therefore, causes demineralization of the skeleton, the extent and rate of which depend upon the quantity of the chelating agent and on the speed of its infusion rather than on the state of the skeletal system (normal, osteolytic or osteoblastic). At no time was the fecal calcium increased, indicating that the excess calcium excretion occurred mainly through the kidney. A maximum of 72 per cent of the theoretic value was excreted as excess in the 24 hours following the infusion. It can be assumed that the unaccounted balance is excreted in small amounts on the following days. The presence of a non-precipitable calcium in the serum 24 hours following the infusion supports this assumption. This is in agreement with the observation that the excretion of C^{14}-labelled EDTA in animals was 99 per cent within 48 hours.[4]

The high excess calcium excretion following the infusion of the salts of EDTAA and the relatively low excess excretion following the infusion of calcium gluconate is of interest. The excess excretion following the latter depends on the state of the skeletal system and on the metabolism of this substance (10), whereas the excess following the former appears to be independent, as these salts seem to be metabolically inactive.

The surplus calcium excretion following the administration of the calcium salt of EDTAA is of importance because of the exchange of injected calcium with calcium of the body or with metals deposited within bone such as radium, yttrium, and lead (2–4). These are chelated by EDTAA preferentially to calcium. The removal of these metals from the bone by exchange is of theoretical and practical importance.

The effects of the calcium salt upon calcium exchange and of sodium salt upon demineralization were studied in man by means of Ca^{45} and will be reported in a subsequent publication (5).

[4] Foreman, Harry. Personal Communication.

SUMMARY

1. The effect of the sodium and calcium salts of ethylenediamine tetra-acetic acid upon the calcium metabolism was studied in ten and five patients, respectively.

2. Slow intravenous infusion of the sodium salt of this agent induced excess calcinuria without lowering the serum calcium levels. This indicates prompt replenishment of the complexed serum calcium by ionized calcium mobilized from the skeletal depots.

3. The calcium salt of ethylenediamine tetra-acetic acid is rapidly excreted through the kidneys.

4. The ability of this agent to remove bone salts and metals deposited in the bone has been discussed.

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PHYSIOLOGICAL EFFECTS OF MECHANICAL EXSUFFLATION ON EXPERIMENTAL OBSTRUCTIVE BREATHING IN HUMAN SUBJECTS[1]

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(Submitted for publication June 5, 1952; accepted September 12, 1952)

The increased physical effort necessary to breathe through a constricted orifice results in an instantaneous and uncomfortable sensation of dyspnea. The immediate sensation of difficulty in breathing is aroused by proprioceptive reflexes. The duration of inspiration and expiration becomes markedly prolonged, (1, 2) and in spite of the increased work entailed, the peak volume flow rates diminish. The minute ventilation has been shown by Cain and Otis (1) to decrease. Haldane and Priestley (3) and others (1, 4) also noted that hypoxia and retention of carbon dioxide develops due to the prolonged duration of the respiratory cycle and reduced alveolar ventilation, resulting from obstructed breathing. Furthermore, a lower or more negative intrapleural pressure develops during the inspiratory phase when breathing against a resistance. The pathological consequences of obstructed breathing, namely pulmonary congestion, have been demonstrated in animals by Moore and Binger (4, 5) and Barach and his associates (6–8).

During the initial studies on the physiological effects of exsufflation, the principle of which is described in Methods, it was noted that the physical effort required to breathe against a resistance introduced at the mouth was markedly diminished (9, 10). Since exsufflation appeared subjectively to relieve dyspnea it was of interest to study its physiological effect on experimentally produced obstructed breathing in normal human subjects. The purpose of this paper is to show the effect of exsufflation on *1*) inspiratory and expiratory volume flow rates, *2*) alveolar pCO_2 and pO_2, and *3*) peripheral venous pressure and intraoral pressure during obstructed breathing.

[1] Aided by a grant from the National Foundation for Infantile Paralysis.

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METHODS

Two mechanical methods of simulating a cough in man were originally devised by Barach and his coworkers (9–11) as a means of eliminating secretions in patients who are unable to cough effectively. One of these methods, exsufflation, *i.e.*, rapid movement of air from the lungs, is accomplished by enclosing the body of the patient in a conventional tank respirator. A negative intra-tank pressure of 40 mm. Hg, developed over a two-second period by a motor blower unit attached to the respirator, is followed by a rapid return to atmospheric pressure in 0.06 seconds by means of a swift-opening valve. The termination of the intra-tank pressure in 0.06 seconds removes a force which tends to keep the lungs inflated in other forms of intermittent pressure breathing where there is a gradually descending expiratory pressure curve (see Figure 1). The rate of exsufflation can be adjusted to produce any desired minute ventilation. This procedure has produced peak expiratory volume flow rates measuring 60 per cent of those

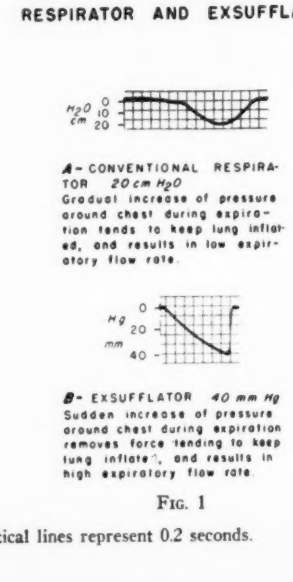

PRESSURE CURVES WITH
RESPIRATOR AND EXSUFFLATOR
H2O cm
0
10
20
A- CONVENTIONAL RESPIRATOR 20 cm H2O
Gradual increase of pressure around chest during expiration tends to keep lung inflated, and results in low expiratory flow rate.
Hg mm
0
20
40
B- EXSUFFLATOR 40 mm Hg
Sudden increase of pressure around chest during expiration removes force tending to keep lung inflated, and results in high expiratory flow rate.

FIG. 1

Vertical lines represent 0.2 seconds.

found in the most vigorous cough obtainable in normal human subjects (9, 11).

Young healthy volunteers who exhibited no evidence of cardio-respiratory disease participated in this study. The subjects were placed in the modified tank respirator, and after the breathing pattern had become regular, control tracings were obtained on a recording spirometer in which the resistance was minimal. Immediately after the control period, obstructed breathing was produced by the introduction of a 3 mm. orifice at the mouthpiece for 6 minutes. During the latter three minutes of this period of obstructed breathing exsufflation was applied at a rate of 9 times per minute.

Determinations of inspiratory and expiratory volume flow rates, alveolar pCO_2 and pO_2, peripheral venous pressure, and intraoral pressure were performed during normal quiet breathing, after the three minute period of obstructed breathing, and at the end of the period of exsufflation.

The spirographic tracings obtained on a high speed drum during normal breathing and obstructed breathing before and after the addition of exsufflation were analyzed in terms of volume of air movement per unit time. The inspiratory and expiratory volume flow rates were derived from these data and plotted graphically.

End expiratory samples of gas were collected and analyzed by the micro-Scholander method. From these data, the alveolar pO_2 and pCO_2 were calculated.

Peripheral venous pressure was recorded from the antecubital vein through an 18-gauge needle attached to a differential Statham strain gauge which was exposed to the intra-tank pressure. Intermittent infusion of heparinized saline assured the fluidity of the system and prevented clotting at the needle. Mean venous pressure was determined by planimetric integration of the area under the pressure curve.

The intraoral pressure tracings were obtained with a 20-gauge needle inserted into the mouthpiece and recorded by a Statham strain gauge exposed to the intra-tank pressure in a manner similar to that of the venous pressure.

The peripheral venous pressure was found to rise with an increase in the mean applied intrapulmonary pressure by Barach and his co-workers (8, 12), Otis, Rahn and Fenn (13) and Barach, Fenn, Ferris and Schmidt (14). Furthermore, Elisberg, Goldberg and Snider (15) noted a positive correlation between intraoral pressures and intrapleural pressures. Under static conditions at the end of inspiration or expiration, pleural pressure equals mouth pressure minus the pressure resulting from lung elasticity. During inspiration or expiration, these relationships are altered by the pressures which are required to overcome the dynamic resistances involved in moving gas and deforming tissue. The measurements of the variations in peripheral venous pressure and intraoral pressure thus served to reflect similar directional changes in the intrapleural pressure.

TABLE I

Obstructed breathing: the reversal of altered inspiratory volume flow rate by exsufflation

Case No.	Maximum volume flow rate: Normal breathing	Maximum volume flow rate: Obstructed breathing	Maximum volume flow rate: Obstructed breathing and exsufflation	Change from obstructed breathing
	cc./sec.	*cc./sec.*	*cc./sec.*	*per cent*
1	605	346	951	+175
2	649	605	735	+ 20
3	605	519	908	+ 75
4	476	432	692	+ 60
5	843	908	1103	+ 21
6	476	345	692	+101
7	476	432	476	+ 10
8	605	435	950	+118
Aver.	592	503	813	+ 72

RESULTS

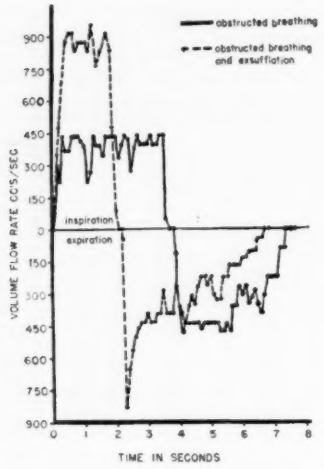
In all subjects the sensation of dyspnea experienced when breathing through a 3 mm. orifice was relieved by exsufflation.

The added resistance to breathing resulted in a 15 per cent decrease in the peak inspiratory volume flow rate. The use of the exsufflator produced a striking increase in volume flow rates averaging 72 per cent above the rate observed during obstructed breathing. This reversal of altered inspiratory volume flow rate is shown in Table I. While breathing against a resistance, the peak expiratory volume flow rate decreased, averaging 28 per cent of that observed during nor-

TABLE II

Obstructed breathing: the reversal of altered expiratory volume flow rate by exsufflation

Case No.	Maximum volume flow rate: Normal breathing	Maximum volume flow rate: Obstructed breathing	Maximum volume flow rate: Obstructed breathing and exsufflation	Change from obstructed breathing
	cc./sec.	*cc./sec.*	*cc./sec.*	*per cent*
1	605	432	692	+ 60
2	778	432	432	0
3	519	346	519	+ 50
4	778	432	519	+ 20
5	584	519	1492	+187
6	519	432	345	− 20
7	648	476	385	− 20
8	519	480	825	+ 72
Aver.	619	444	651	+ 43



OBSTRUCTED BREATHING: EFFECT OF EXSUFFLATION ON VOLUME FLOW RATE

Maximum Volume Flow Rate	Inspiration	Expiration
Obstructed Breathing	435 cc	480 cc
Obstructed Breathing With Exsufflation	950 cc	825 cc

FIG. 2

mal expiration. A rise in expiratory volume flow rate of 43 per cent above the obstructed breathing level was induced by exsufflation, as seen in Table II. When the expiratory volume flow rate was plotted against time, as is demonstrated in Figure 2, the maximal increase in flow rate was found to occur at the onset of expiration.

The average tidal volume during normal breathing was 581 cc., and fell to 485 cc. or by 16 per cent when breathing against a resistance. As is seen in Table III, when exsufflation was added the average tidal volume was 1,074 cc., a rise of 85 per cent over that in normal breathing and 121 per cent over that during obstructed breathing.

TABLE III

Tidal air volume

Case No.	Normal breathing	Obstructed breathing	Obstructed breathing and exsufflation
	cc.	*cc.*	*cc.*
1	735	395	1,513
2	393	432	1,211
3	670	660	1,103
4	411	419	886
5	751	778	935
6	389	246	1,124
7	468	535	951
8	432	412	876
Aver.	581	485	1,074

The average minute ventilation of 7,431 cc. fell to 5,328 cc. or by 27 per cent during obstructed breathing. During exsufflation, minute volume was 9,644 cc. or 29 per cent higher than during normal breathing, and 81 per cent above that during obstructed breathing. This is shown in Table IV.

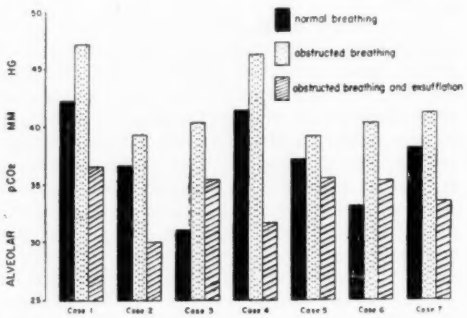
TABLE IV

Minute ventilation, cc./min.

Case No.	Normal breathing	Obstructed breathing	Obstructed breathing and exsufflation
		cc. min.	
1	9,555	6,320	13,617
2	6,288	4,616	10,899
3	8,710	7,920	9,927
4	6,165	6,447	7,974
5	9,012	7,780	8,505
6	6,184	4,182	10,016
7	7,488	6,420	8,559
8	6,048	4,944	7,884
Aver.	7,431	5,328	9,644

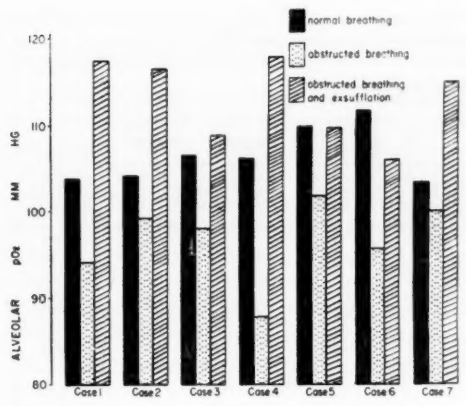
In seven subjects the average alveolar pCO_2 rose 4.8 mm. Hg or 13 per cent (range 5.4 to 29.9 per cent) during obstructed breathing. The addition of exsufflation resulted in a reversal of CO_2 tension toward, and in some instances below, the control level. These findings are illustrated in Figure 3. The average alveolar pCO_2 produced by exsufflation was 8.0 mm. Hg or 19 per cent (range 11.2 to 31.4 per cent) below that present during obstructed breathing.

During obstructed breathing, the alveolar pO_2 fell an average of 9.7 mm. Hg or 9.1 per cent



OBSTRUCTED BREATHING: THE EFFECT OF EXSUFFLATION ON ELEVATED ALVEOLAR pCO_2

FIG. 3



OBSTRUCTED BREATHING: EFFECT OF EXSUFFLATION ON LOWERED ALVEOLAR pO_2

FIG. 4

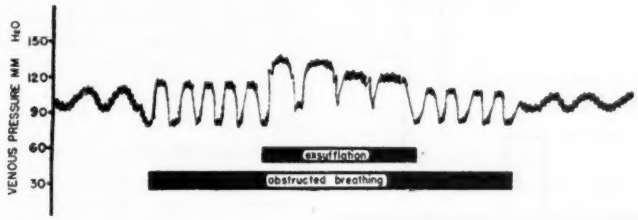
(range 3.2 to 17.3 per cent) below the control values in seven subjects. Following 3 minutes of exsufflation the pO_2 increased an average of 12.4 mm. Hg or 16.9 per cent (range 7.8 to 34.2 per cent) above that observed with obstructed breathing. This is graphically illustrated in Figure 4.

The peripheral venous pressure was determined in eight subjects and recorded relative to the tank pressure. The average mean venous pressure fell 7 mm. H_2O when a resistance to breathing was introduced. The addition of exsufflation resulted in an average rise of 20 mm. H_2O above this level and 13 mm. H_2O above the normal breathing level, as seen in Table V. During the inspiratory phase an average drop of 6 mm. H_2O (range 3 to 16 mm.) was noted when the obstruction to breathing was added. The minimal level recorded during exsufflation was 12 mm. H_2O above the value obtained during obstructed breathing and 6 mm. H_2O above the normal breathing value. A

TABLE V

Obstructed breathing: the reversal of altered mean venous pressure by exsufflation

Case No.	Mean venous pressure (mm. H_2O) Normal breathing	Obstructed breathing	Obstructed breathing and exsufflation
1	83	77	87
	72	69	79
2	106	100	112
	81	72	101
3	98	79	90
	99	91	99
4	97	92	113
5	70	63	78
	64	52	64
6	78	73	110
7	74	72	84
8	68	62	106
	78	71	108
Aver.	82	75	95



OBSTRUCTED BREATHING: EFFECT OF EXSUFFLATION ON VENOUS PRESSURE

	Minimum	Maximum
Normal Breathing	87mm	108mm
Obstructed Breathing	78mm	113 mm
Obstructed Breathing With Exsufflation	90mm	123mm

FIG. 5

The lowering of the inspiratory venous pressure produced by obstructive breathing is abolished during exsufflation.

TABLE VI

Obstructed breathing: the reversal of altered intraoral pressure by exsufflation

Case No.	Intraoral pressure (mm. H_2O)					
	Minimal level recorded			Maximal level recorded		
	Normal breathing	Obstructed breathing	Obstructed breathing and exsufflation	Normal breathing	Obstructed breathing	Obstructed breathing and exsufflation
1	−6	−43	+4	0	+58	+112
2	−2	−84	+4	+1	+66	+139
3	−3	−36	+6	0	+36	+143
4	−2	−45	+3	+1	+31	+149
5	−5	−52	+2	+2	+35	+143
6	−5	−87	+1	0	+53	+124
Aver.	−4	−58	+3	+0.6	+47	+135

representative venous pressure tracing, shown in Figure 5, reveals that the lowered negative pressure during inspiration is no longer present when exsufflation is added.

An average negative intraoral pressure of 58 mm. H_2O relative to the tank pressure was recorded when breathing through a 3 mm. orifice. The addition of exsufflation resulted in a striking reversal of this negative intraoral pressure. From data presented in Table VI and Figure 6 the lowest pressure observed with exsufflation was plus 1 mm. H_2O.

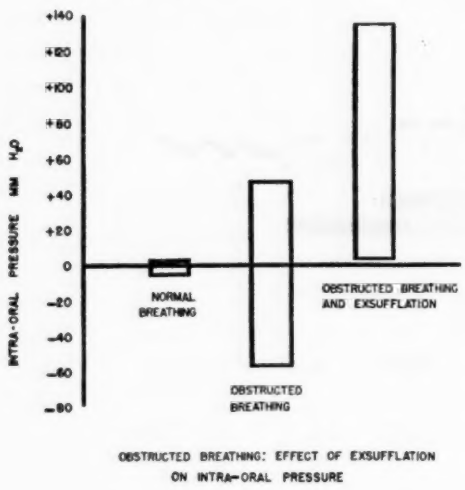


FIG. 6

The lowering of the inspiratory intraoral pressure produced by obstructed breathing is abolished during exsufflation.

DISCUSSION

An attempt has been made in this study to simulate as much as possible the obstructive component present in clinical conditions which constrict the larynx, trachea or bronchi, including tumor mass, foreign body, or the thick viscid bronchial secretions associated with bronchial asthma, bronchiectasis and the bronchospastic type of pulmonary emphysema. In normal respiration the bronchial tree lengthens and dilates during inspiration and less resistance or obstruction to breathing is therefore present in this phase of breathing than in the expiratory cycle. In the present study the added resistance to breathing remained constant during both phases of respiration and therefore a pathologic intrabronchial obstruction to breathing is not entirely reproduced.

In animal experiments it has been shown by Moore and Binger (4, 5) and Barach (6–8) that obstructed breathing either during inspiration or both phases of respiration results in pulmonary congestion and intra-alveolar edema. These changes were not found when a comparable resistance to breathing was maintained during expiration only. The pathological consequences of obstructive breathing were therefore attributed to the abnormally lowered pressure exerted on the lungs during inspiration. The sequelae of obstructive respiration appear to be traced to the effect of increased negative pressure on pulmonary capillaries, production of mucus from the bronchial mucous membrane, an increased output of the right heart, and a diminished output from the left heart. In cases of severe bronchial asthma, a

pathologically lowered negative intrapleural pressure during inspiration has also been demonstrated by Barach (16).

Maloney and Whittenberger (17) and Beck, Seanor and Barach (18) have shown that the physiological effects of negative pressure surrounding the body in a tank respirator are comparable to those produced by positive pressure applied at the mouth and nose. This relationship had already been pointed out by Henderson (19). The mechanism of exsufflation, therefore, includes inspiratory positive pressure breathing, which is mainly comparable to positive pressure breathing produced in other ways. In studies by Motley and his co-workers (20, 21) on intermittent positive pressure breathing it was found that the type III curve with a low mean pressure and a rather rapid return to atmospheric pressure in expiration was the respirator of choice. However, in the type III curve expiration lasts 1.11 seconds and the return to atmosphere takes place in approximately 0.5 seconds. In exsufflation the mean applied pressure is 5.5 cm. H_2O and expiration, except for the first 0.06 seconds, takes place at atmospheric pressure. The relaxation pressure of the lungs, therefore, operates more effectively during the greater part of the expiratory cycle. Furthermore, the relative absence of increased intrapulmonary pressure during the expiratory cycle of exsufflation permits the use of high ventilatory pressures with minimal effect on the circulation. Since the effect on cardiac output has been shown to depend on the mean applied intrapulmonary pressure (14) and rapidity of drop of expiration pressure (20, 21), exsufflation with its low mean pressure and rapid drop of pressure at the beginning of expiration probably has little effect on the mean output of the heart.

The introduction of a resistance to breathing, resulting in increased inspiratory and expiratory effort produces physiological effects comparable to negative pressure breathing in inspiration and positive pressure breathing in expiration. The addition of inspiratory positive pressure by exsufflation more than compensates for the inspiratory negative pressure breathing, and inspiration now takes place under positive pressure. Since with exsufflation expiration (except for the first 0.06 seconds) takes place at atmospheric pressure, the relaxation pressure of the lungs during this procedure overcomes the effect of resistance to air flow during the expiratory cycle. Thus, exsufflation during obstructed breathing results in positive pressure breathing during both phases of respiration.

It has been demonstrated (1, 2) that the inspiratory flow rate is less frequently disturbed in obstructed breathing than is that of expiration. In our studies, peak volume flow rate decreased 15 per cent during inspiration and 29 per cent during expiration. The alteration of volume flow rate produced by exsufflation was most strikingly manifested in inspiration. The expiratory volume flow rate was increased particularly at the onset of expiration. The major action of exsufflation on the volume flow rate during inspiration is explained by the fact that air is being introduced into the lungs under positive pressure. The increased flow rate at the onset of expiration can be attributed to the rapid elimination of the negative pressure surrounding the chest, allowing the relaxation pressure of the lungs and surrounding tissues to operate promptly and at its fullest extent.

In current studies by Bickerman, Beck and Barach (22) the diameters of medium-sized bronchi of dogs are increased two-fold at the peak of the 40 mm. Hg pressure. With the abrupt termination of this pressure there is a rapid contraction and shortening of the entire bronchial tree, which is a factor in the production of the high volume flow rate seen especially at the onset of expiration.

The advantageous effect of exsufflation in relieving the hypercapnia and hypoxia which develop during obstructed breathing is clearly demonstrated. Exsufflation would appear to be of special value in patients with pulmonary emphysema who have developed respiratory acidosis, since it would provide an effective method of restoring to normal the altered CO_2 and O_2 tensions present in this syndrome.

Cain and Otis (1) have shown that the total ventilation decreases while breathing against an added resistance. In our studies the fall in tidal volume and minute ventilation during obstructive breathing was markedly reversed by the addition of exsufflation. The rise in oxygen tension during exsufflation despite a rate of only nine times a minute must be due to a more effective alveolar ventilation. Since the rate of exsufflation can be

controlled, any desired increase in minute ventilation can easily be achieved without the high mean intrapulmonary pressures produced by conventional forms of pressure breathing, where there is ventilation with a gradually descending expiratory pressure curve.

The marked drop in peripheral venous pressure and intraoral pressure during the inspiratory phase of obstructed breathing is overcome by exsufflation and there is at no time the development of lowered intraoral or peripheral venous pressure. Assuming that intraoral and peripheral venous pressure are reflections of intrathoracic pressures, an abnormally lowered negative intrapleural pressure is present during the inspiratory phase of obstructed breathing, and a reversal of this state takes place with the addition of exsufflation. A reduction in the height of negative intrapleural pressure by the application of pressure breathing has also been shown by Barach (*7*) in experimentally induced obstructive dyspnea in dogs and in a patient with severe bronchial asthma. He also showed that as the intrapleural pressure became more negative, pulmonary congestion developed, and that anything that decreased the negative intrapleural pressure would tend to decrease the likelihood of development of congestion. Since obstructed breathing over a prolonged period of time will result in pulmonary congestion, the physiologic action of exsufflation in reversing the lowered venous pressure and presumably the lowered intrapleural pressure would appear to prevent the consequences of obstructive dyspnea and thus be useful in clinical entities characterized by obstructive lesions in the respiratory tract.

SUMMARY

Obstructive respiration was produced in normal human subjects by breathing through a 3 mm. orifice. Marked subjective dyspnea was accompanied by the following pathophysiological consequences: *1*) A diminution of inspiratory and expiratory volume flow rates; *2*) An elevation of alveolar pCO_2 and lowering of pO_2; *3*) A lowering of peripheral venous pressure; and *4*) A marked decrease in inspiratory intraoral pressure.

Exsufflation consists of inflation of the lungs with a 40 mm. Hg pressure and a rapid termination of the pressure at the onset of the expiratory cycle. When this mechanism was applied during obstructed breathing, subjective dyspnea was relieved and the physiological alterations noted were reversed towards normal levels. The beneficial effect of exsufflation can be attributed to (*a*) inspiratory positive pressure breathing, and (*b*) the prompt and effective use of the increased relaxation pressure of the lungs.

The restoration towards normal of physiologic consequences of experimentally induced obstructed breathing suggests that the use of the mechanism of exsufflation would be of therapeutic value in those clinical conditions characterized by obstructive lesions in the respiratory tract.

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STUDIES ON DESOXYRIBONUCLEASE IN SYSTEMIC LUPUS ERYTHEMATOSUS. NON-PARTICIPATION OF SERUM DESOXYRIBONUCLEASE IN THE "L.E. PHENOMENON" [1]

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In 1948, Hargraves, Richmond, and Morton (1) described a new cellular element in the bone marrow of patients with acute systemic lupus erythematosus. This element, referred to as the "L.E. cell," has been confirmed by a number of investigators, and has been suggested as a diagnostic aid (2).

The L.E. cell may be formed during the incubation of normal homologous or heterologous leucocytes (3) with the sera of some patients suffering from lupus erythematosus. Haserick, Lewis, and Bortz (4) have shown that the serum factor responsible for the phenomenon resides in the gamma-globulin fraction. The L.E. cell is a polymorphonuclear neutrophilic granulocyte which contains a homogeneous, basophilic inclusion in its cytoplasm. Commonly associated with this phenomenon is the formation of rosettes (clusters of polymorphonuclear leucocytes about a large homogeneous basophilic mass) and "globs" (free homogeneous basophilic masses, approximately the size of a white blood cell). The inclusions and extracellular masses have been shown to contain desoxyribonucleic acid (DNA) in a depolymerized form (5). The hematoxylin-stained bodies described in tissues of mesodermal origin by Gross (6), Ginzler and Fox (7), and Klemperer, Pollack and Baehr (8), have also been demonstrated to contain depolymerized DNA (9a, b). Since as far as is known this nucleic acid occurs, normally, exclusively as a constituent of the chromosomes, it may be concluded that the homogeneous bodies that characterize the L.E. cell phenomenon are nuclear in origin.

The DNA of normal nuclei, unlike that of the homogeneous masses characterizing the L.E. cell phenomenon, is highly polymerized (10). The participation of the specific depolymerizing enzyme, desoxyribonuclease (DNase), in the production of the L.E. cell has therefore been suggested (9b, 11). This enzyme occurs in the blood serum of many animals (12–15) and appears to be a constituent of all cells (16), including those of bacteria (17, 18) and yeast (19). The purpose of this communication is to report investigations of the possible role of the serum DNase [3] (as opposed to intracellular DNase) in the production of the L.E. phenomenon. Previous observations on human serum DNase (15) provide control data for these studies.

METHODS

A. *Patients.* Twenty-three patients with systemic lupus erythematosus were studied.[4] The severity of the disease process was estimated according to the presence and intensity of toxicity, fever, joint involvement, rash, anemia, cardiac and renal involvement, and rapidity of eryth-

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[3] Preliminary studies by Dr. S. Zamenhof in collaboration with one of the authors (S. L. L.) suggested abnormal serum DNase activity in systemic lupus erythematosus.

[4] We wish to acknowledge the kindness of the staff of the Roosevelt Hospital, New York City, for permitting us to study a patient in that hospital. We are indebted, also, to Dr. John Haserick of the Cleveland Clinic for providing us, in the Spring of 1950, with sera from three patients with systemic lupus erythematosus. Most of the patients were studied on the Medical Services of the Mount Sinai Hospital, New York.

rocyte sedimentation rate. The composite clinical picture was graded arbitrarily from 0 to 4+.

B. *Estimation of serum DNase activity.* Venous blood was drawn from the subjects in the fasting state, allowed to clot, and the serum separated within one hour. Merthiolate (Lilly), which has no effect on DNase activity in concentrations as high as 1:1000 (15), was added as a preservative at a concentration of 1:100,000. When not used immediately, the sera were stored at −20° C. The DNase activity of the sera was determined by the methyl green method (11), slightly modified (15).

C. *Quantitation of the "L.E. cell" phenomenon.* A quantitative modification of the clot technique for demonstrating the L.E. cell phenomenon (20) was performed as follows. At the same time that blood was drawn for DNase determination, 5 ml. of blood was drawn into a 15 ml. centrifuge tube and allowed to clot. The clot was agitated and removed with a wooden applicator. The tube was centrifuged and the supernatant serum decanted. The sediment was resuspended in the approximately 0.5 ml. serum which remained in the tube. The suspension was incubated at 37° C. At intervals of 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 24 hours, drops of the suspension were smeared on glass slides, fixed with methyl alcohol, and stained with dilute Giemsa stain. The slides were labelled with a code. To assure objectivity, all slides were examined as unknowns by a single observer who was not familiar with the code. The results were recorded as the percentage of involved cells in 500 consecutive polymorphonuclear neutrophilic granulocytes. Criteria for involvement were either the presence of the classical L.E. cell (*i.e.*, a leucocyte with a single large cytoplasmic inclusion), or a granulocytic leucocyte containing droplet inclusions of nuclear material. Alternatively, slides were graded from 0 to 4 on the basis of the frequency of occurrence of each of the three characteristic changes: (a) L.E. cells (including those with droplet inclusions), (b) rosettes, (c) "globs." Maximal intensity of these changes was noted between the second and fourth hours of incubation and this value was recorded as a measure of the "L.E. phenomenon activity" of the serum.

The two grading systems are not directly comparable, since the percentage scheme does not provide for weighting of rosettes and "globs."

D. *Leucocyte suspensions.* These were prepared by a slight modification of the method of Minor and Burnett (21), by drawing blood from normal subjects into tubes containing 10 mg. heparin per 50 ml. blood. A 50 mg./ml. solution of bovine fibrinogen (Fraction I, Armour), previously dialyzed against 0.9 per cent saline to remove sodium citrate, was added (1 ml. fibrinogen solution per 25 ml. whole blood) to hasten erythrocyte sedimentation. The blood was permitted to sediment by gravity at room temperature and within one hour the supernatant leucocyte-rich plasma was pipetted off. Leucocyte counts were performed on this plasma, and appropriate aliquots containing 5 to 10 million white blood cells were transferred to small serological test tubes, centrifuged, and the supernatant cell-free plasma decanted, leaving "cell buttons" of known size. The cell buttons were then used as indicators for the L.E. cell phenomenon by resuspension and incubation at 37° C. for one hour in solutions of the various serum fractions described below (E). "Buttons" were also suspended in (a) 1 ml. 0.14 M NaCl containing 1 mg. crystalline bovine pancreatic DNase (22) [5] and 0.003 M $MgSO_4$, (b) 1 ml. containing DNase and $MgSO_4$ as in (a) plus 2 mg. polymerized beef thymus DNA (23), and (c) 1 ml. 0.14 M NaCl containing 1 mg. DNA. "Buttons" were also suspended in 1 ml. fresh serum from patients with lupus erythematosus and with 1 ml. serum previously heated to 60° C. for 10 minutes. Aliquots of both the heated and unheated sera were tested for DNase activity.

TABLE I

Electrophoretic analysis of serum protein fractions prepared from serum of patient W. G.

	γ-Globulin	β-Globulin	α-Globulin	Albumin
	%	%	%	%
Serum	27	19	21	33
Fraction II	99			
Fraction III-A		46	54	
Fraction III	*	*	*	
Fraction IV-1		7	90	3
Fraction IV-4		9	29	62
Fraction V				100

* Solution was too turbid for analysis, but small amount of γ-globulin was indicated.

E. *Fractionation of serum from a patient with systemic lupus erythematosus.* A phlebotomy of 500 ml. was performed on patient W. G. two weeks after transfusions and cortisone therapy had been discontinued, and again one week later. After clotting, the sera were promptly separated and frozen. The 700 ml. of serum were pooled and fractionated by the Cohn Method 6 (modified) (24) and Method 9 (modified) [6] (25). The following fractions were obtained: II, III-A, III, IV-1, IV-4, and V. Their composition is given in Table I. Aliquots were reconstituted in saline to five times their concentration in the original serum. These were tested for DNase activity in the same manner as sera. The ability of each fraction to produce the L.E. cell phenomenon was also tested, using buttons of 5 to 10 million leucocytes and 1 ml. of the serum fraction solution incubated for 1 hour at 37° C. In the latter study, each fraction was present in a final concentration four times that of the original serum.

RESULTS

The observations on the estimation of DNase and L.E. cell provoking activities of the sera and

[5] Worthington Labs., Freehold, N. J.

[6] We are indebted to Dr. John W. Palmer and Mr. Leo Zuckerman of the Research and Development Laboratories of E. R. Squibb & Sons, New Brunswick, New Jersey, for the preparation of these fractions and the electrophoretic analyses in Table I.

TABLE II

Summary of clinical data, serum "L.E. phenomenon activity" and DNase activity

Subject and date	Age and sex	Clinical severity	ACTH or Cortisone	Serum "L.E. phenomenon activity"	DNase $\times 10^{-4}$†
				*per cent**	*units/ml.*
H. S. Oct. 31, 1950	12 M	+++	0	21	124
July 25, 1951		+++	ACTH	75	41
R. B. Oct. 10, 1950	30 M	+	ACTH	27	52
Jan. 9, 1951		+	ACTH	18	0
July 31, 1951		++	ACTH	64	28
R. V. Oct. 10, 1950	19 F	+	ACTH	4	73
Jan. 2, 1951		+	ACTH (1 yr.)	10	67
July 31, 1951		+	0 (3 mos.)	54	55
E. McI. Aug. 23, 1950	39 F	++++	0	29	79
Oct. 17, 1950		++++	0	5	111
Jan. 9, 1951		+	Cortisone	1	66
Aug. 2, 1951		++	0	75	157
C. Jan. 8, 1951	47 F	+	ACTH (6 mos.)	1	71
O. Jan. 9, 1951	18 F	±	0	0	48‡
F. W. Nov. 14, 1950	13 M	+	ACTH	2	66
W. G. July 19, 1951	25 F	+++	0	50	11
July 25, 1951		+++	0	60	31
R. K. July 25, 1951	12 F	++	ACTH	25	41
Aug. 20, 1951		++	ACTH		181
E. L. July 25, 1951	35 M	+	0	26	41
C. B. July 25, 1951	36 F	+	0	43	42
S. K. July 25, 1951	60 F	+++	ACTH	31	41
Aug. 18, 1951		++++	ACTH	weak	92
M. F. July 25, 1951	41 F	++	ACTH	58	14
E. K. July 25, 1951	15 F	++	ACTH	3	21
F. H. July 31, 1951	32 F	+	0	37	85
N. M. Aug. 2, 1951	25 F	++++	ACTH	67	89
G. J. July 31, 1951	22 F	+++	0	59	123
L. R. July 31, 1951	25 M	++	0	30	74
				G. *L.E.* *R.*§	
H. Oct. 23, 1951	30 F	+	0	0 2 ½	34
A. Oct. 23, 1951	47 F	+	0	1 2 1	44
L Oct. 23, 1951	35 F	++	0	0 1 2	86
E. B. Oct. 16, 1951	13 F	++	0	1 3 2	49
Nov. 31, 1951		++	0	1 3 3	50
W. W. Jan. 25, 1951	37 F	++++	0	1 2 1	56
Feb. 9, 1951		++++	Cortisone	2 3 3	338

* Graded in per cent of polymorphonuclear neutrophilic granulocytes transformed to "L.E. cells."
† See Kurnick (11) for definition of unit.
‡ Spleen (removed for treatment of thrombocytopenia) showed changes characteristic of Lupus erythematosus.
§ Graded in arbitrary units 0 to 4.
G. "Globs"
L.E. "L.E. cells"
R. Rosettes

the clinical state of the patients are presented in Table II. The correlation coefficients (r), and their probabilities of significance (P) between each pair of parameters are listed in Table III.

TABLE III

Statistical analysis of correlations of data of Table II

Correlations	r	P
"L.E. phenomenon activity" and serum DNase activity	.511	.0045
"L.E. phenomenon activity" and clinical severity	.423	.0158*
Serum DNase activity and clinical severity	.362	.0162*

* Doubtful significance.

The L.E. phenomenon activity and serum DNase activity each reveal low order correlations of doubtful significance with the clinical severity of the disease. Although a significant correlation between serum DNase and L.E. phenomenon activity exists, it is of such a low order as to have no practical value for prediction.

The serum DNase activities of patients with systemic lupus erythematosus cover a range from 0 to 338×10^{-4} units/ml. with a mean of 73 ± 10 (standard error), compared to the normal range of $0–119 \times 10^{-4}$ units/ml. with a mean of 51 ± 3.5 (15). The difference between the means is probably significant ($P = 0.0091$). A total of 168 determinations of the DNase activities of sera from patients with lupus erythematosus were made. Only 36 of these are included in Table II and in the calculation of the mean DNase activity, because of incomplete data on L.E. phenomenon activity or clinical state for the remaining values. If all 168 values are included, the mean serum DNase activity in acute systemic lupus erythematosus becomes $67 \pm 3.1 \times 10^{-4}$ units/ml., which is significantly higher than the normal mean ($P = 0.0002$), but not significantly different from the mean value calculated from the smaller series.

The incubation of leucocytes with bovine DNase with or without added polymerized DNA (in the absence of serum containing the L.E. factor) produced no visible alteration in the leucocyte morphology. However, if the serum with L.E. phenomenon activity was also present, the cytoplasmic inclusions of the L.E. cells were either very pale-staining or appeared as unstained vacuoles. This effect is presumably due to the dissolution of the partially depolymerized DNA (which is responsible for the basophilia) of the inclusions by the bovine DNase.

None of the fractions prepared from the sera of patient W. G. (see E above) revealed any DNase activity. Fraction II produced a strong L.E. cell phenomenon (86 per cent of polymorphonuclear neutrophilic leucocytes involved) while Fraction III was very slightly active (less than 1 per cent of polymorphonuclear neutrophilic granulocytes involved). None of the other fractions produced any L.E. cells.

Serum DNase was almost completely inactivated by heating for 15 minutes at 60° C. (15). However, such heated serum produced the L.E. cell phenomenon with little or no impairment in intensity (Table IV).

TABLE IV

Effect of heating serum for 10 minutes at 60° C on "L.E. phenomenon activity"

Serum	Treatment	"Globs"	L.E. cells	Rosettes	Total
R.V.	Unheated	3	4	4	11
R.V.	Heated	1	4	4	9
M.F.	Unheated	1	4	4	9
M.F.	Heated	2	4	3	9

* Intensity of reaction was scored on basis of 1 to 4.

DISCUSSION

The observation that bovine DNase is without effect on unfixed human leucocytes is in agreement with the observations of Ely and Ross (26) that surviving cells are not susceptible to the enzyme, whereas the DNA of dead cells is readily degraded. Presumably, viable cells are not permeable to the enzyme. The incubation of leucocytes with polymerized DNA alone, or in the presence of bovine DNase, does not induce changes resembling those of the L.E. cell. This indicates that the L.E. cell is not produced simply by the phagocytosis of DNA liberated from cells lysed in a hypothetical preliminary stage of the phenomenon.

The localization of the L.E. factor in Cohn's Fraction II (27) was confirmed. In agreement with reports (3–5) that the L.E. factor is associated with the serum gamma-globulin, Fraction II, which represents the bulk of the gamma-globulin, and Fraction III, which contains a small amount

of gamma-globulin (Table I), were the only serum fractions found to contain L.E. phenomenon activity.

The facts that serum fractions with strong L.E. cell provoking activity were devoid of DNase activity, and that sera rendered almost free of DNase activity by heat remained potent L.E. cell producers, demonstrate that the L.E. factor and serum DNase are not directly associated. The correlation found between DNase activity of the fresh serum and its L.E. phenomenon activity, is, therefore, not a causal relationship.

Although these studies indicate that serum DNase is not responsible for the L.E. cell phenomenon, subsequent studies (28–30) suggest the participation of intracellular DNase and a DNase inhibitor in the depolymerization of DNA which characterizes this phenomenon.

SUMMARY

1. The role of serum desoxyribonuclease (DNase) in the production of the depolymerization of desoxyribonucleic acid (DNA) characteristic of the "lupus erythematosus (L.E.) cell" of Hargraves was studied. It was found that serum DNase is not responsible for the phenomenon.

2. Serum DNase activity in systemic lupus erythematosus tends to be slightly higher than normal, and displays a low order of correlation with the "L.E. phenomenon activity" of the serum. The low order correlation found between serum DNase activity and the clinical state of the patient is of doubtful significance.

3. Serum DNase is less stable to heat than the serum factor which provokes the L.E. cell transformation.

4. The L.E. cell phenomenon cannot be induced by the incubation of normal leucocytes with bovine DNase with or without added DNA.

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OXYGEN TENSION OF TISSUES BY THE POLAROGRAPHIC METHOD. V. THE RATE OF MOVEMENT OF OXYGEN FROM THE PERIPHERAL ARTERY TO THE SKIN [1]

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By means of the oxygen electrode (1), which measures polarographically the oxygen tension of living tissues *in situ*, several workers have reported a time lag in the changes of tissue oxygen after arterial blood oxygen changes. Behrmann, Griest, Mangun, and Hartman showed in hypoxemia studies in dogs that the changes in oxygen tension of animal viscera lagged measurably behind the changes of oxygen in the peripheral arterial blood (2). Experiments have been made with pressure cuffs in human extremities. On deflating a cuff the oxygen tension of the skin of the extremity recovered some time after the skin became fully pink (3). In another set of human experiments, changes in skin oxygen tension lagged considerably behind the changes in blood arterial oxygen saturation (4).

In none of these studies was the lag between the *initial* changes of arterial and tissue oxygen compared. It is the purpose of this report to describe this time lag by comparing the exact time of first appearance of oxygen in skin tissue (polarographic measurement) with the exact time of first appearance of oxygen in the peripheral arterial blood (oximetric measurement) and to correlate this "saturation-tension lag" with gross changes in peripheral blood flow. The increment in blood oxygen saturation was furnished by inhalation of oxygen by the subject.

METHODS

Subjects were either white healthy adults or white patients with peripheral arterial disease. A Millikan oximeter was attached to the ear and "arterialization" of the blood in the ear was accomplished by heat, in the usual manner (5). Oxygen electrodes were inserted in the skin of one or more extremities (3). Estimates of skin blood flow were made by taking the temperature of the skin adjacent to the electrodes and of air in the room, all obtained by means of thermocouples. Room temperature was constant throughout each experiment. Some of the experiments were conducted in a thermoregulated "cool room." Oxygen-administering apparatus consisted of a demand valve attached to a mouth-piece through a three-way stop-cock.

After the readings of skin temperature, of oxygen electrode and of oximeter became stable, the nose of the subject was obstructed by a clip, the valve and attached tubing were flushed out with oxygen, oxygen was administered by quickly turning the stop-cock just prior to the beginning of a normal inspiration and "zero" time was set with the first "hiss" of the demand valve. The very first changes of the readings by oximeter and by oxygen electrode were obtained by noting the first increment in current shown by each galvanometer. Subsequent changes were noted at approximately five-second intervals for a period of at least one minute. In order to make frequent oxygen tension readings the electrode switch (3) was kept continually closed, starting one minute prior to the beginning of oxygen inhalation.

RESULTS

A. *Warm skin, with normal blood flow*

For convenience, measurements of the oxygen tension were usually made in the forearm. When made in the hand, foot or digits, the tension lag did not differ significantly unless blood flow was different.

Most of the subjects were normal individuals. A few were patients with peripheral arterial disease who had approximately normal peripheral arterial blood flow as measured by skin temperature. The average temperature of the skin of extremities of all the subjects was 31.7° C., and of the room 24.6° C. Heat was added to the body, if needed, to maintain the skin temperature of the extremity at the desired level.

In these experiments the first change of the arterial oxygen saturation in the ear, occurred, as expected (6), in an average of 8.6 seconds after the start of oxygen inhalation. The first change in the oxygen tension (polarographic method) of

[1] This work was supported by the Life Insurance Medical Research Fund.

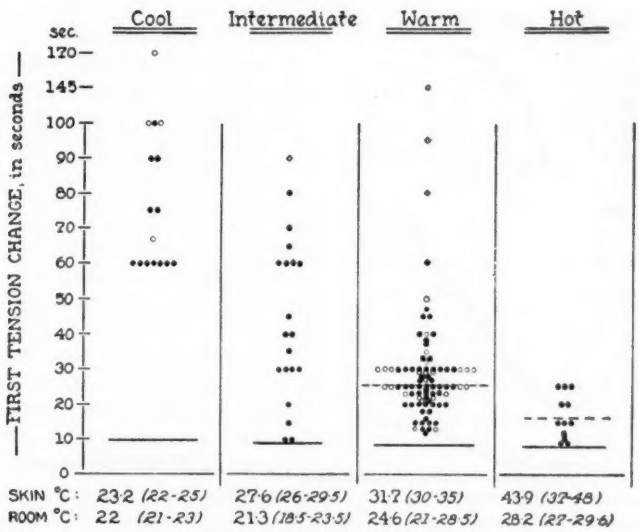

FIG. 1. THE FIRST CHANGE IN OXYGEN TENSION OF COOL, INTERMEDIATE, WARM AND HOT SKIN; ALL EXPERIMENTS

Empty symbols indicate observations before November 17, 1950; solid symbols, after that date (see text).

◊, ◆ indicate that no oxygen tension change had taken place by this time and that the experiment was carried no further. Such values were not used to derive mean values.

– – – is mean value of first oxygen tension change, as measured in the skin of an extremity by polarographic method.

——— is mean value of first oxygen saturation change, as measured by the oximeter.

Temperature of HOT skin is that of the water when direct heating was obtained by immersion in water bath (see Figure 5).

the warm skin of the forearm, however, took place in an average of 25.5 seconds, lagging behind the first change of the arterial blood by 16.9 seconds (Figures 1, 2).

Special mention should be made of the early group of experiments on normal subjects (done before November 17, 1950 in which an occasional, considerably longer delay of the first change in tension was noted. In three of these 28 experiments, the first change in the oxygen tension of the skin was very slow and indefinite, not occurring within 60 seconds. Possibly the way of inserting the electrodes was responsible for the results in these three instances. These 28 early experiments are shown in Figure 1. In all subsequent 57 experiments (after November 17, 1950), when special attention was given to the insertion of the electrodes in the skin, only one such unusually long delay was encountered (Figure 1).

B. *Cool skin, with reduced blood flow*

To investigate the role of peripheral blood flow on the time interval between change in oxygen saturation of arterial blood and in oxygen tension of skin, measurements were made in the skin of extremities of six patients with peripheral arterial disease, and of one normal subject, before and after reflex vasodilatation caused by heating the body. Before vasodilatation, the first change of the arterial saturation following oxygen inhalation took place in an average of 9.8 seconds, but the first change of the oxygen tension of the cool skin was considerably delayed. When the skin temperature was 23.2° C., and the room temperature

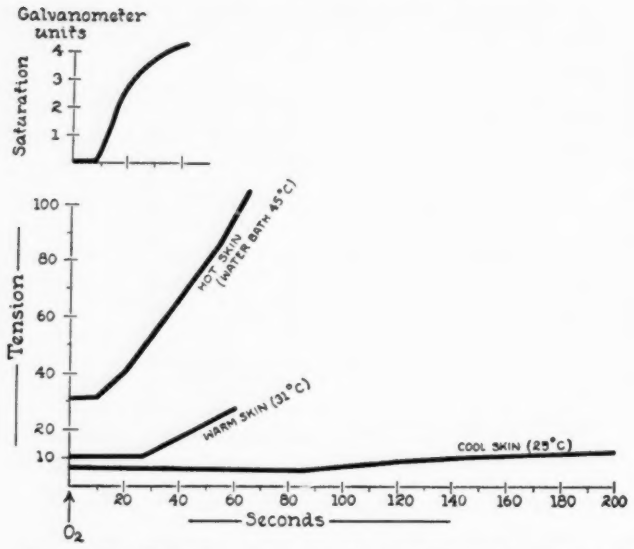

FIG. 2. THE FIRST CHANGE IN THE ARTERIAL OXYGEN SATURATION OF THE BLOOD AND IN THE OXYGEN TENSION OF WARM, COOL, AND HOT SKIN. THREE TYPICAL EXPERIMENTS

Saturation changes are essentially the same in all experiments. "O_2" indicates beginning of inhalation of oxygen. The experiments are on three different subjects.

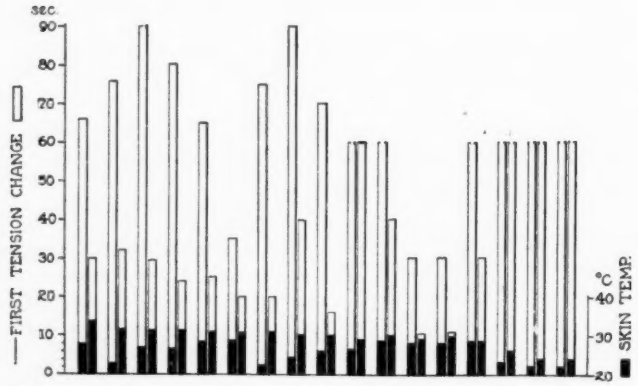

FIG. 3. THE FIRST CHANGE IN OXYGEN TENSION OF THE SKIN BEFORE AND AFTER REFLEX VASODILATATION

Each pair of bars represents values obtained with one electrode, remaining undisturbed throughout the experiment. The first bar of each pair represents the value obtained before reflex vasodilatation was attempted by body warming; the second bar, the value after body warming. Broken line at top of bar means experiment was terminated at this time without a skin oxygen tension change having as yet been detected.

22° C., a first tension change failed to occur within 60 seconds. In those instances, after body warming, in which the previously cool skin of an extremity became warm by reason of reflex vasodilatation an average time of 26 seconds was necessary for the first change of the oxygen tension. In those patients in whom a significant degree of reflex vasodilatation failed to occur, and the skin remained cool, the time necessary for the first change of the oxygen tension remained over 60 seconds; with incomplete vasodilatation (skin temperature from 26 to 29.5° C.), the first tension change was variable (Figures 1, 2, 3).

Studies before and after body warming of a patient with symptoms of ischemia in the left leg as a result of occlusion of the popliteal artery are shown in Figure 4. The other extremities were unaffected. As blood flow changed, or remained unchanged as in the case of the left leg, corresponding changes in the time lag resulted.

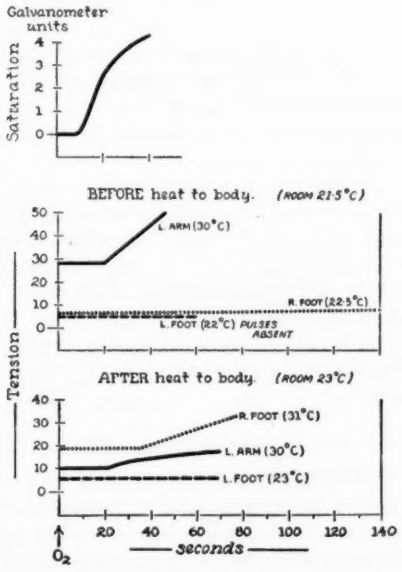

FIG. 4. THE FIRST CHANGE OF THE BLOOD ARTERIAL SATURATION AND OF THE OXYGEN TENSION OF THE SKIN, BEFORE AND AFTER REFLEX VASODILATATION, BY HEAT TO BODY, IN A PATIENT WITH PERIPHERAL ARTERIAL DISEASE OF THE LEFT LEG (OCCLUSION OF LEFT POPLITEAL ARTERY)

Saturation changes, shown in the upper graph, are essentially the same before and after reflex vasodilatation.

C. *Hot skin, with extremely rapid blood flow*

To investigate the role of extremely rapid peripheral blood flow on the saturation-tension lag, the hands of several normal subjects, having reflex vasodilatation, were further heated by directly applying hot air or hot water. As the heat was increased the lag was reduced (Figures 1, 2, 5). In five hot air experiments, air temperature was maintained at a minimum of 50° C., raising the skin temperature from 35 to 44° C. (average). The first change in the oxygen tension of the skin took place in an average of 21 seconds. In the three hot water experiments, water with average temperature of 43.9° C. was used. The first change in the oxygen tension of the hot skin took place in an average of 12 seconds. In two of the latter experiments in which the water temperature was 47° C., oxygen appeared in the skin in 9 and 11 seconds, almost simultaneously with its detection by the oximeter, on the ear.

In no case was the time of the first oxygen saturation change altered significantly from that in previous experiments, averaging 8 seconds.

D. *Further investigation of the saturation-tension lag*

Several small groups of experiments were performed to investigate further the factors responsible for the time lag in the appearance of oxygen in the skin after its appearance in the peripheral arterial blood:

1. *Time required for oxygen to travel from the capillaries to the electrode tip.* In the preceding experiments the saturation-tension lag indicates the time required for oxygen to travel through the distal portion of the peripheral artery, arterioles, capillaries and extravascular tissue to the tip of the oxygen electrode. A few experiments were, therefore, carried out to determine what portion of this time was required for the movement of oxygen from the capillaries to the electrode tip. When a blood pressure cuff is inflated on an arm to above systolic pressure for approximately 10 minutes, the skin loses its pink color and the oxygen in the hand is much depleted. When the cuff is released the skin becomes pink as a result of oxygenated blood entering the cutaneous capillaries. The oxygen tension rises shortly thereafter to its original value (3).

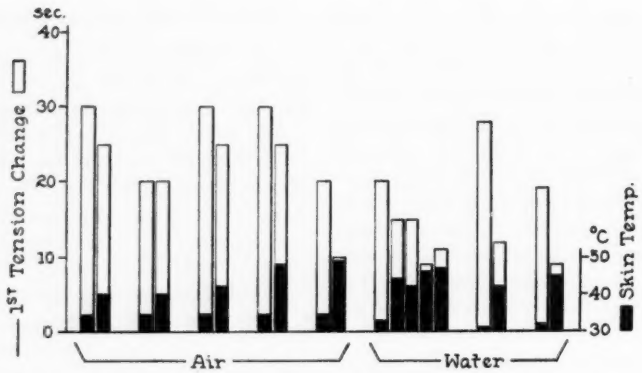

FIG. 5. THE FIRST CHANGE IN OXYGEN TENSION OF THE SKIN BEFORE AND AFTER DIRECT HEATING OF THE SKIN

Eight experiments are represented. Each group of bars represents the values of one experiment, with the electrode remaining undisturbed throughout the experiment. The first bar of each group shows the value in the warm, vasodilated skin, before direct heating. The remaining bar (or bars) are the values obtained after direct heating of the skin. "Air," heated by hot air; "Water," by hot water.

With this in mind, three electrodes were placed in the skin of fingers. A blood pressure cuff on the upper arm was inflated for ten minutes and suddenly released. In three experiments on the same subject, pink color was clearly apparent in 2 to 2.5 seconds, whereas the first change in tension required 10, 12, and 25 seconds, respectively, in the three electrodes. Later in these same experiments, with electrodes undisturbed, and with no arterial compression, oxygen was inhaled (usual experiment) and the increase in oxygen tension of the skin occurred in 15, 15, and 30 seconds, respectively.

In two other experiments, oxygen was inhaled for several minutes while a cuff was inflated, and the cuff was then released. The inhalation of oxygen did not change the lag; the pink color appeared in 4 seconds, whereas the first increase in tension required 27 and 34 seconds after release of the cuff.

2. *Effect of changing temperature of skin in a leg with ischemia.* An experiment was conducted in order to study the effect of changes in skin temperature, *per se*, on the saturation-tension lag. A severely ischemic foot, whose greatly reduced capacity for blood flow had been demonstrated by a reflex vasodilatation test (skin temperature equal to room temperature) and by posterial tibial nerve block, was tested at room temperature (27° C.), and after immersion in water at 37° C. At both temperatures the lag remained greater than 60 seconds.

3. *Inhalation of a low oxygen gas mixture.* In three experiments, the first changes of the oxygen tension of the warm normal skin after the inhalation of 10 per cent O_2 were compared with those after the inhalation of pure O_2. The time of first change after the low oxygen inhalation (15, 25, and 45 seconds) was approximately the same as that after inhalation of pure O_2 (20, 23, and 45 seconds).

E. *Incidental observations (unrelated to the saturation-tension lag)*

1. *The degree of increase of oxygen tension of cool, vasoconstricted skin with continued oxygen inhalation.* Since the oxygen tension of such skin fails to increase within 60 seconds of oxygen inhalation (Figure 1), two extended experiments were conducted on cool skin. One lasted for six minutes, with skin temperature 22.5° C., room temperature 22° C. The oxygen appeared in the skin in 75 seconds and the percentage increase at the end of two and six minutes was 16 per cent

and 33 per cent, respectively. At the end of an experiment lasting ten minutes, on another subject with skin temperature 22° C., and room temperature 23° C., oxygen appeared in the skin in 100 seconds and the percentage increase at the end of two and one-half minutes and ten minutes was 6 per cent and 30 per cent, respectively.

2. *Increase of oxygen tension of reflexly vasoconstricted and iced skin with continued oxygen inhalation.* In one experiment the skin temperature of reflexly vasoconstricted skin of the foot of a normal subject was further lowered to less than 15° C. by the direct application of ice, producing the bright pink color characterized by Lewis as the red reaction to cold (7). Oxygen inhalation for five minutes failed to cause any change in oxygen tension.

3. *Increase of oxygen tension of hot, vasodilated skin with continued oxygen inhalation.* In three experiments there was a great increase in oxygen tension of skin under the combined effects of reflex vasodilatation and direct application of heat and oxygen inhalation. In two instances in which the water temperature was 47° C., the oxygen tension was increased with oxygen inhalation by 75 per cent in 30 and 40 seconds. In a third experiment, water temperature 45° C., it was increased by 218 per cent in one minute (Figure 2).

4. *A comparison of oxygen tension of vasoconstricted and vasodilated skin.* In six experiments in which cool skin was warmed from an average temperature of 25° C. to 30.5° C. by peripheral vasodilation resulting from heating the body (room temperature 22° C.), the oxygen tension of the skin was increased approximately by 270 per cent of the initial value, as in experiments previously reported (3).

DISCUSSION

The time of arrival of the first increment of oxygen in the blood of the great arteries of an extremity is probably very nearly the same as the time of arrival at the oximeter on the ear. The great arteries of an extremity are a little more distant from the heart than is the ear, but the increment shown by the oximeter measures the oxygen increment in ear capillaries, which may be a second or so later than that in the carotid artery. The average first increment of arterial oxygen saturation detectable by the oximeter following the first inhalation of oxygen, was about 9 seconds throughout all experiments. Our measurements indicate that oxygen entered the skin some 16 seconds after its appearance in the arterial blood of the ear when the skin was comfortably warm, and its circulation correspondingly fast. This delay occurred in such warm skin whether the subjects were normals or were known to have spastic or occlusive peripheral arterial disease if reasonably fast circulations were maintained in the extremity.

This observation shows that there is a significant, and sometimes conspicuous, delay in the movement of oxygen from the great vessels of an extremity to the skin of the extremity. Under these various conditions, therefore, the time required for oxygen to travel with the blood in the large arteries of an extremity, through the arterioles and capillaries and into the skin tissue is about twice the time required for its entrance into alveoli, diffusion into pulmonary capillaries, its travel through pulmonary vein, left heart, carotid artery, and its entrance into the dilated minute vessels of the ear.

Reflex vasoconstriction in an extremity delayed the first appearance of oxygen in the skin of the extremity far beyond 16 seconds. It did not affect the time of first appearance of oxygen in the great vessels. Consequently, the greatest part of the delay in movement of oxygen in the extremities seems to have occurred in the minute vessels, capillary walls, and extravascular tissues. Further evidence that this is the site of delay comes from experiments in which peripheral vasomotor tone was greatly reduced by a combination of reflex vasodilatation and extreme direct heating. With the minute vessels widely dilated, oxygen entered the skin of the extremities some two seconds after its first appearance in the ear.

The few experiments described in "D. 1." of "Results" were attempts to learn degrees of delay in the extravascular tissue in contradistinction to degrees of delay in the minute vessels. When the cuff was released, oxygenated blood was visible in fingers in about two seconds whereas its detection by the electrode was at least six seconds later. This delay of six seconds is explainable only by time taken for diffusion of oxygen from minute vessels to the electrode. This diffusion time was studied only in warm skin under the particular set

of circumstances, and was not studied further. Doubtless it varies with the number and size of open capillaries, just as the delays of oxygen transport within vessels vary with vasomotor tone.

SUMMARY

1. The rate of movement of oxygen was studied by administering pure oxygen by inhalation and noting its very first appearance in the peripheral arterial blood and in the skin.

2. Its appearance in the arterial blood was measured by means of the Millikan oximeter attached to the ear, and in the skin of an extremity by means of the oxygen electrode.

3. Blood flow to the skin of the extremities was altered over a wide range in normal subjects and in patients with peripheral arterial disease.

4. The delay in the movement of oxygen from the peripheral artery to the warm vasodilated skin (average 16 seconds) was markedly increased in cool, vasoconstricted skin, and was conspicuously decreased in hot, extremely vasodilated skin. Evidence is cited that the greatest part of the delay in the movement of oxygen occurs in the minute blood vessels, capillary wall and/or extravascular tissue.

5. In a few related experiments, in warm vasodilated skin, it was also shown that (a) a considerable portion of the delay was due to the time required for oxygen to travel from the capillary, through the extravascular tissue, to the tip of the electrode; (b) 10 per cent oxygen inhalation was followed by the same changes as pure oxygen; and (c) the temperature change of the skin, accompanying blood flow change, did not, *per se*, have any great affect on the rate of movement of oxygen.

6. Finally, some incidental observations on the effect of continued oxygen inhalation on the percentage rise of the oxygen tension of reflexly vasoconstricted, directly chilled, and directly heated skin are reported.

ACKNOWLEDGMENT

The authors would like to express their appreciation for the assistance rendered by Miss Diane Grabosky and Miss Ann Sayen.

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INTRAVENOUS TRYPSIN: ITS ANTICOAGULANT, FIBRINOLYTIC AND THROMBOLYTIC EFFECTS[1]

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Fibrinogenolysis and prolonged blood coagulation have been observed following large intravenous doses of crystalline trypsin (1).[2] The present study was undertaken to evaluate further the effects of intravenous crystalline trypsin upon the protein components of the coagulation mechanism and the fibrin content of artificially induced intravascular thrombi, in 60 rabbits and 25 dogs. A striking observation in this study was the lack of untoward reactions, including shock, when the rate and concentration of injected trypsin were kept within carefully calculated limits.

MATERIALS

1) Sixty rabbits whose average weight was 2 kg. were fed rabbit food pellets and given water *ad libitum*.

2) Twenty-five mongrel dogs, including 5 dogs used as controls, were housed together and fed Purina Dog Chow. Their average weight was between 10 to 12 kg.

3) Crystalline trypsin, *i.e.* Armour Tryptar® preparation,[3] was used in each experiment.

4) 0.5 ml. of 10 per cent sodium morrhuate, or 0.2 ml. (125 units) of topical thrombin (Parke, Davis), or 0.5 ml. of thromboplastin (simplastin) were used to produce thrombi in rabbits and dogs.

5) Bovine fibrinogen (Armour), and topical thrombin (Parke, Davis) were used in antithrombin determinations.

METHODS

1) *Experimental production of thrombi in rabbits and dogs.* Experimental thrombi were produced in one of the marginal ear veins of each of 60 rabbits by injecting 0.5 ml. of 10 per cent sodium morrhuate or 125 units of topical thrombin or 0.5 ml. thromboplastin into the vessel. The distal portion of the vein was then compressed for 5 to 10 minutes. Experimental thrombi were produced in the femoral vein of 10 dogs. Under Nembutal® anesthesia, the femoral vein was exposed in the upper third of the thigh without dividing the femoral fascia. Then 0.5 ml. of 10 per cent solution of sodium morrhuate was injected into the vein. During the injection, and for 5 to 6 minutes thereafter, the vessel was gently compressed distal to the point of the injection. A firm thrombus, verified by palpation and dissection of the vessel, formed 10 to 15 minutes following instillation of the thrombogenic agent.

2) *Preparation of trypsin solution.* In experiments on rabbits, 250,000 units of specially prepared trypsin (250 mg. of tryptic activity) were dissolved in 250 ml. of isotonic saline. A vinylite catheter was introduced into the ear vein, and the inflow was regulated at 6 drops per minute. In experiments on dogs, 1,000,000 units of trypsin were dissolved in 500 ml. of isotonic saline, and introduced through the femoral vein. The infusion rate was maintained at 35 to 40 drops per minute.

3) *Laboratory determinations.* All prothrombin determinations were done according to the method of Quick (2). Fibrinogen was determined by the method of Greenberg (3). The Innerfield, Angrist, and Benjamin method (4) of antithrombin titer determination was used. Factor V was measured by the method of Stefanini (5); and fibrinolysis assay was made by the Conley, Ratnoff, and Hartmann method (6). Determinations of coagulation time were made by the Lee-White method (7). Blood volume was estimated following administration of labeled albumin (8). Continuous measurements of clotting viscosities were made with a Rich-Roth Ultra-viscosimeter (9).

PROCEDURE

1) *Rabbits.* Blood samples were obtained from non-anesthetized rabbits by cardiac puncture, before and at varying intervals during infusion of trypsin. Sections of an ear vein thrombus were obtained for histologic study prior to, during, and after completion of each intravenous infusion.

2) *Dogs.* Trypsin infusions were given to dogs who had been anesthetized with 6 per cent Nembutal. Blood samples were obtained prior to, and during the course of the infusion. In securing

[1] Aided by research grant from The Armour Laboratories, Chicago, Ill. and the David Schwartz Fund, Jewish Memorial Hospital, New York, N. Y.

[2] On the basis of animal observations crystalline trypsin (Tryptar) was administered intravenously to 101 patients. The only untoward reaction was an occasional mild chill toward the end of an infusion. It appears that, when carefully controlled, intravenously administered crystalline trypsin is a safe procedure. Data on this clinical study constitute a separate report.

[3] The trypsin used in this study was kindly supplied by Marvin C. Ziporyn, M.D., of the Armour Laboratories.

TABLE I

Mean effect of increasing increments of small intravenous trypsin dose upon the coagulation mechanism in 6 dogs

Trypsin units *30 drops/min.*	Prothrombin time *seconds*	Factor V *units*	Fibrinogen *mg. %*	Antithrombin clotting time —5 Min. Incuba.— *seconds*	Coagulation time *minutes*
Control	13	750	360	26	7.6
500	13	710	368	27	7.0
1,000	13	690	354	26	7.1
2,000	13.5	705	373	26	7.5
3,000	14	670	349	27	8.1
5,000	12.5	720	370	26	7.6

plasma, nine volumes of blood were added to one volume of 1.85 per cent sodium citrate; the plasma was carefully removed from the cellular elements and determinations were carried out. Sections of thrombi artificially induced in the femoral vein were obtained from 10 dogs.

RESULTS

The data are summarized in Tables I through V. Following the administration of small doses of trypsin to 6 dogs, no significant changes were noted in the coagulation mechanism (Table I). Control studies included determinations of coagulation time, prothrombin time, factor V, antithrombin clotting time, and fibrinogen. Following the administration of trypsin intravenously in graded doses ranging from 500 to 5,000 units, no appreciable deviation from normal was noted. Following the administration of intravenous trypsin in doses of 25,000 to 250,000 units in rabbits, and 50,000 to 1,000,000 units in dogs, the blood

TABLE IV

Duration of prolonged clotting time following massive intravenous doses of trypsin

Dogs	Trypsin dosage *30 drops/min.*	Clotting time				
		Before treatment	After treatment			
			½ hr.	1 hr.	15 hrs.	24 hrs.
	units	*min.*	*min.*	*min.*	*min.*	*min.*
D	300,000	8	42	7	8	8
B	750,000	6	400+	400+	400+	9
G	1,000,000	8	400+	400+	400+	12

TABLE II

Mean effect of increasing increments of massive intravenous trypsin dose upon the coagulation mechanism, thrombi, and blood volume in 60 rabbits

Trypsin units *30 drops/min.*	Prothrombin time *seconds*	Factor V *units*	Fibrinogen *mg. %*	Antithrombin —5 min. incuba.— *seconds*	Antifibrinolysin	Coagulation time *min.*	Effect upon thrombi	Blood volume %
Control	5	650	350	25	++	3	0	100
25,000	11	610	370	25	++	3.5	0	100
50,000	17	590	340	26	+	2.8	0	98
75,000	22	540	310	24	+	4.6	++	96
100,000	36	410	290	21	0	7.8	++	91
150,000	54	390	210	18	0	15.9	+++	76
200,000	100+	48	55	14	0	60+	++++	51
250,000	100+	31	22	14	0	60+	++++	33

TABLE III

Mean effect of increasing increments of massive intravenous trypsin doses upon the coagulation mechanism, thrombi and blood volume in 25 dogs

Trypsin units *30 drops/min.*	Prothrombin time *seconds*	Factor V *units*	Fibrinogen *mg. %*	Antithrombin —5 min. incuba.— *seconds*	Antifibrinolysin	Coagulation time *min.*	Effect upon thrombi	Blood volume %
Control	14	750	350	25	+ to ++	7.6	0	100
50,000	16	670	365	24	++	7.9	0	96
100,000	19	640	220	26	+	8.2	+	93
200,000	29	590	165	23	+	11.1	++	84
300,000	56	270	70	21	0	42.2	+++	91
500,000	300+	110	50	17	0	150+	++++	86
750,000	300+	too low to assay	too low to assay	16	0	400+	++++	68
1,000,000	300+	too low to assay	too low to assay	16	0	400+	++++	44

TABLE V

Fibrinolysis in 15 dogs at increasing levels of intravenous trypsin

Dogs	Occurrence of fibrinolysis *trypsin units*					
	0	50,000	100,000	200,000	350,000	1,000,000
1	—	—	—	X	X	XX
2	—	—	X	X	XX	XX
3	—	—	—	—	X	XX
4	—	—	X	X	X	XX
5	—	—	X	X	X	XX
6	—	—	—	—	X	XX
7	—	—	—	X	X	XX
8	—	—	—	—	X	XX
9	—	—	X	X	XX	XX
10	—	—	—	—	X	XX
11	—	—	—	X	X	XX
12	—	—	—	—	XX	XX
13	—	—	X	X	X	XX
14	—	—	—	—	—	XX
15	—	—	—	—	X	XX

— No fibrinolysis.
X Fibrinolysis within the first 24 hours.
XX Fibrinolysis within 20 minutes.

coagulation time was prolonged, protein constituents of the clotting mechanism showed diminishing titers, the blood volume was reduced, and changes in the consistancy and microscopic appearance of intravascular thrombi were observed. Quantitatively, these changes roughly paralleled the level of enzyme concentration infused at any given time.

A. *Rabbits*

1) Prothrombin. The magnitude of change in prothrombin time varied from 5 seconds in the control animals to greater than 100 seconds after the administration of more than 200,000 units of trypsin.

2) Factor V. The mean level of factor V in control rabbits was 650 units. Appreciable diminution did not occur until 100,000 units of trypsin had been administered. A precipitous fall occurred when the dose exceeded 200,000 units.

3) Fibrinogen. There was slight variation from the normal value of 350 mg. per cent following the introduction of 100,000 units of trypsin. When 150,000 trypsin units had been administered, a drop to 210 mg. per cent was observed. A profound fall in fibrinogen occurred following doses exceeding 200,000 trypsin units.

4) Antithrombin. Subsequent to the administration of 75,000 trypsin units mean antithrombin titers declined. Marked depression in antithrombin titer occurred when the trypsin dose exceeded 200,000 units.

5) Antifibrinolysin. The phenomenon of antifibrinolysis disappeared following doses in excess of 100,000 trypsin units.

6) Effect upon thrombi. Changes in thrombi were recorded on a 0 to 4 plus basis, varying from no observable change to increasing thrombolytic changes (Figure 1). No appreciable gross or microscopic alterations in thrombi were noted prior to the administration of 75,000 units. Two to three plus changes occurred in the 75,000 to 200,000 trypsin unit range. Complete clot dissolution and restoration of circulatory integrity consistently occurred at a range beyond 200,000 units of trypsin.

7) Blood volume. Significant changes in mean blood volume did not occur prior to the administration of 150,000 units of trypsin. At 200,000 units, however, the blood volume was 51 per cent of control; at 250,000 units the blood volume fell to 33 per cent of control.

8) Coagulation time. The coagulation time showed slight variations prior to the administration of 100,000 trypsin units. Following the administration of additional trypsin, the coagulation time became markedly prolonged. When trypsin doses exceeded 200,000 units, *in vitro* blood samples failed to clot throughout an observation period of 72 hours.

B. *Dogs*

1) Prothrombin time. The prothrombin time showed prompt changes following increasing doses of trypsin. After 100,000 units of trypsin the prothrombin time was 19 seconds; after 300,000 units the prothrombin time was 56 seconds; at and above 500,000 units the prothrombin time was greater than 300 seconds. The control prothrombin time was 14 seconds.

2) Factor V. The control was 750 units. Marked diminution was observed after 300,000 units of trypsin had been introduced. Following trypsin doses exceeding 750,000 units, the quantity of factor V was too low to determine.

3) Fibrinogen. Pronounced decrease in fibrinogen (control level 350 mg.) was observed after the administration of 200,000 units of trypsin. Extreme depression in fibrinogen was observed with 300,000 trypsin units. Quantities too low to

assay appeared when more than 750,000 units of trypsin were administered.

4) Antithrombin. The titer of antithrombin varied inversely with the quantity of injected trypsin. This inverse relationship became most apparent after 500,000 units had been administered. "Absolute" depression in antithrombin titer occurred beyond 750,000 trypsin units.

5) Antifibrinolysin. Following the administration of trypsin in doses greater than 350,000 trypsin units, the phenomenon of antifibrinolysis could not be demonstrated.

6) Effect on thrombi. Lytic changes in thrombi were demonstrable following administration of 100,000 trypsin units.

7) Blood volume. Significant changes in blood volume did not occur until trypsin was administered in quantities exceeding 500,000 units. At 750,000 units of trypsin, the blood volume dropped to 68 per cent of control; at 1,000,000 units the blood volume was 44 per cent of control. Between 300,000 and 500,000 units, the blood volume varied from 91 to 96 per cent respectively.

8) Coagulation time. The coagulation time showed only moderate prolongation prior to the administration of 300,000 units of trypsin. Inordinately prolonged blood coagulation time followed doses exceeding 500,000 units of trypsin. Blood samples obtained one half-hour after 300,000 unit infusions of trypsin showed a mean coagulation time of 42 minutes. Samples obtained one hour after the administration of 300,000 units of trypsin, however, showed normal coagulation time. Following the administration of trypsin in a

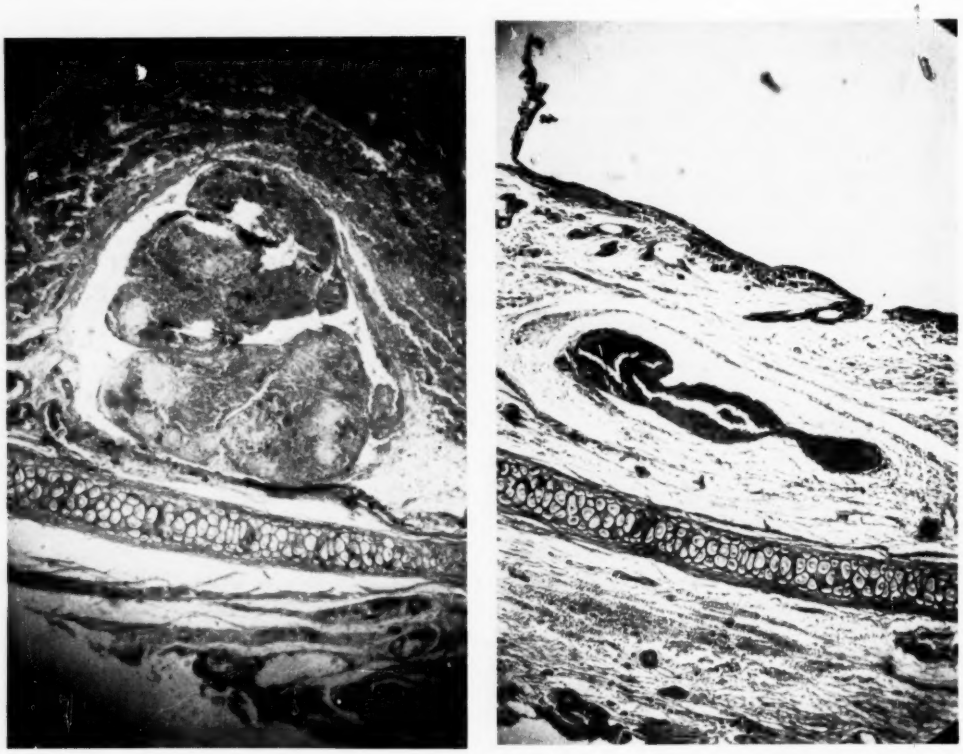

FIG. 1. HISTOLOGIC SECTIONS OF EXPERIMENTALLY PRODUCED EAR VEIN THROMBUS IN RABBIT TAKEN

(A) Immediately following completion of injection of sodium morrhuate, and

(B) Twenty-four hours after intravenous administration of 200,000 Armour Units of trypsin in 250 ml. of isotonic saline, given at the rate of six drops per minute into the marginal vein of opposite ear.

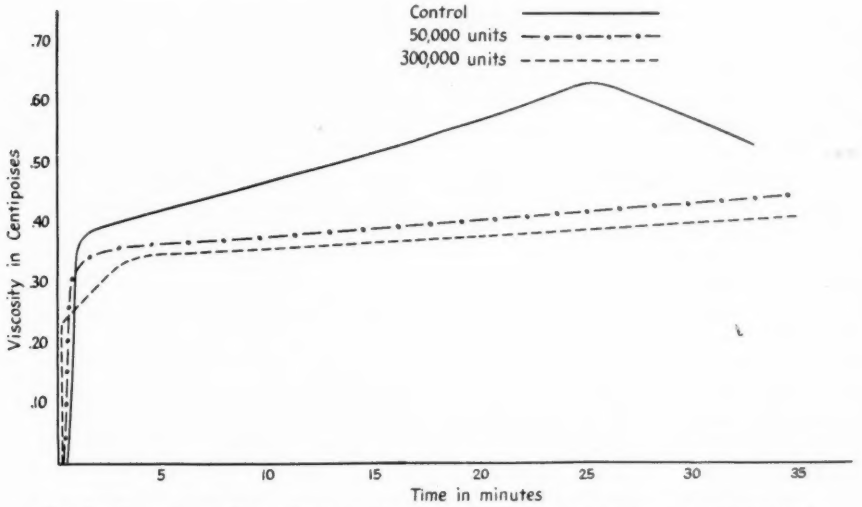

FIG. 2. CHANGES IN BLOOD CLOTTING VISCOSITY FOLLOWING INTRAVENOUS ADMINISTRATION OF TRYPSIN TO A DOG

750,000 to 1,000,000 unit range, the coagulation time was greatly prolonged in random blood samples obtained during a 15 hour period of observation. At the end of 24 hours, the clotting time showed only slight prolongation.

9) Fibrinolysis. There was no evidence of fibrinolysis prior to the administration of 50,000 units of trypsin. Samples obtained from five of 15 dogs showed evidence of fibrinolysis at the 100,000 units level. Samples obtained from eight of 15 dogs showed fibrinolysis at the 200,000 trypsin unit level, and 14 of the 15 dogs showed fibrinolysis after 350,000 trypsin units. Each sample tested showed fibrinolysis when 1,000,000 units was administered.

10) Blood clotting viscosity. A sharp decrease in blood clotting viscosity occurred following the administration of 50,000 units of trypsin to one dog. More marked diminution in viscosity was evident after the administration of 300,000 units to the same animal (2).

DISCUSSION

Activation of proteolytic enzyme systems (fibrinolysis) has been produced by diversified methods in the past two decades. During the course of *in vitro* experiments this phenomenon followed: (*a*) the conversion of plasminogen to plasmin by culture filtrates of Streptococci hemolyticus (streptokinase) (10), (*b*) the removal of plasmin inhibitor by certain organic solvents, such as chloroform and ether (11), (*c*) the activation of proplasmin into plasmin by a "Factor" from mammalian tissue (12), (*d*) the conversion of an inactive precursor enzyme present in culture filtrates of group A streptococci into a proteinase by autocatalysis or by the action of trypsin (13), (*e*) the action of pancreatic trypsin upon plasminogen causing the release of plasmin (14, 14a) and (*f*) the action of trypsin upon fibrin (15).

Although there is convincing evidence that trypsin produces fibrinolysis *in vitro,* our observations on the fibrinolytic effects of intravenous trypsin do not necessarily prove a direct trypsin-fibrin substrate interaction. Trypsin may resemble streptokinase in its capacity to activate a fibrinolytic system by transforming naturally occurring plasminogen into plasmin (14, 14a). Whether plasmin then acts synergistically with trypsin, or the fibrinolytic effects following intravenous trypsin are predominantly due to the liberation of plasmin requires further evaluation.

There have been reports that intravenous trypsin caused shock (16), intravascular coagulation (17, 19) local necrosis (18), sloughing of tissue (18), and was generally "too toxic for intravenous use"

(19). The appearance of shock in our experiments was conditioned by such critical factors as infusion rate, enzyme concentration, and total dose. In a previous study, shock developed within 15 seconds following a rapid intravenous injection of trypsin in a dose of only 2,000 units/kg. (1). In the present investigation, administration of the enzyme solution at *6* to *8* drops per minute in rabbits, and *30* to *40* drops per minute in dogs did not produce shock for several hours during the course of a single infusion, despite massive enzyme concentrations and dose, *i.e.*, 125,000 units/kg. in rabbits and 100,000 units/kg. in dogs. Occasionally, shock did appear when doses exceeding 150,000 units of trypsin were given to rabbits, or greater than 750,000 units were given to dogs.

We could not confirm the observation that a small dose of intravenous trypsin hastens blood coagulation or predisposes toward intravascular thrombosis (19). Following administration of trypsin in the same small dose range previously associated with intravascular clotting (100 to 200 units/kg.) (20), the coagulation time, prothrombin time, factor V, antithrombin and fibrinogen values were normal (Table I). It is conceivable that circulating antitryptic substances adequately inactivate small quantities of injected trypsin. In order to rule out the possibility that multiple thrombi followed small trypsin doses despite normal findings of the circulating constituents measured, seven rabbits and three dogs were sacrificed following small intravenous trypsin doses. After extensive dissection, no evidence of intravascular thrombosis was noted either grossly or microscopically.

Marked anticoagulant effects followed large intravenous doses of trypsin. When the dose of trypsin was steadily increased (continuous intravenous infusion) a point was reached beyond which all clotting proteins were proteolyzed (Tables II and III). In such extreme instances the blood became incoagulable presumably because the protein constituents of the coagulation mechanism had been depolymerized to amino acids.

The lytic effect of intravenous trypsin upon experimentally produced thrombi was most impressive. Gross changes in the rabbit ear vein thrombi following intravenous trypsin included *1*) diminution and in several instances disappearance of the thrombus *in situ*, *2*) restoration of local circulation, and *3*) restoration of vessel wall compressibility. The microscopic features of trypsin treated thrombi included *1*) diminution of fibrin, *2*) diminution of cellular and platelet masses, and *3*) no untoward effect upon vessel walls. Fibrinolytic effects extended into the laminations and interstices of the thrombus.

Gross changes were not observed in control animals with artificially induced clots who were given intravenous saline. Furthermore, it was noted microscopically, in these saline-treated control animals, that there was a persistence of the dense fibrin meshwork, plus homogenous masses of platelets and cells.

It appears probable, therefore, that the intravenous route effectively provides surface contact between proteolytic enzymes and the fibrin meshwork in thrombi, and that lysis of artificially formed thrombi in rabbits and dogs was produced by virtue of fibrinolytic mechanisms activated by intravenous trypsin. On the basis of these experimental data, the enzymatic action of *in vivo* trypsin upon the coagulation mechanism, and its effect upon fibrin and clots *in situ*, merits further study and elucidation.

SUMMARY

1. Under controlled conditions of infusion rate, enzyme concentration, and dosage, the intravenous administration of trypsin is safe and feasible in rabbits and dogs.

2. Small trypsin doses *in vitro* produce clots. The effects of small trypsin doses *in vivo* is apparently nullified by circulating antitryptic substances.

3. Large trypsin doses both *in vitro* and *in vivo* have powerful anticoagulant effects.

4. Intravenously administered trypsin induces lytic effects upon artificially formed intravascular thrombi in rabbits and dogs.

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SOME HORMONE STUDIES IN NORMAL AND TOXEMIC PREGNANCY [1]

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The steroids of the adrenal cortex assist in, and are necessary for, normal excretion of water and electrolyte (1). In general, these steroids tend to cause water excretion and salt retention, the relative amounts of these responses being dependent upon the structure of the steroid. The hormones of the posterior pituitary gland, on the other hand, cause water retention and, usually, sodium and chloride excretion (1). A reciprocal relationship between these hormones has been proposed (2).

Since the suggestion by Hofbauer (3) on theoretical grounds that posterior pituitary hormones might play a part in the development of eclampsia and the report by Anselmino, Hoffmann, and Kennedy (4) of an increased level of an antidiuretic material in the plasma of toxemic patients, a large number of studies of the role of antidiuretic materials in toxemia have been carried out. Some of these studies have failed to demonstrate an antidiuretic substance or have failed to show a correlation between antidiuretic activity and toxemia of late pregnancy. The failures to find increased antidiuretic activity have been mostly when antidiuretic activity has been sought in serum or plasma (5–10). A good many workers have been able to show an increased urinary antidiuretic activity (11–13) or an increased sensitivity to exogenous posterior pituitary hormone in patients with pre-eclampsia or eclampsia (10, 14–17).

Several studies of corticosteroid excretion during pregnancy have found the level of these steroids to be increased in toxemia (18–21). It has recently been demonstrated in normal non-pregnant subjects and in patients with abnormalities of water excretion that during a state of positive water balance, a relatively high level of antidiuretic activity in serum and a relatively low urinary corticosteroid excretion are found; conversely, in the presence of a negative water balance, relatively low levels of antidiuretic activity and high levels of corticosteroid occur (22). No simultaneous studies have been made during pregnancy of antidiuretic and adrenal cortical substances. Therefore, a study of the possible role of these factors in the water metabolism of normal and toxemic pregnancies was undertaken. Because pregnanediol and chorionic gonadotrophin excretion are also altered during toxemia (23–26), it was felt worthwhile to attempt to correlate the urinary excretion of these materials with the other measurements.

METHODS

Twenty-four hour urine specimens were refrigerated during collection or, when refrigeration was impossible, were preserved with chloroform. All of the determinations were carried out as soon as possible after completion of the collection, usually within two or three hours. Pregnanediol was measured by the method of Guterman and Schroeder (27). Chorionic gonadotrophin was measured by the method of Behnken, Lloyd, and Hughes (28). For this method, 1 rat unit equals from 2½ to 3 international units of chorionic gonadotrophin. Corticosteroid was determined by the method of Lloyd and Lobotsky (29). For all specimens the "total corticosteroid" was measured. For many specimens the "freely-water-soluble corticosteroid" and the "poorly-water-soluble corticosteroid" were also determined. In almost all non-pregnant women, the "freely-water-soluble corticosteroid" is essentially equivalent to the "total corticosteroid" and very little "poorly-water-soluble corticosteroid" is found. The formaldehyde formed is converted to milligrams of corticosteroid by multiplication by a factor of 11.5 (30). The range of normal values for non-pregnant women of the same age as those studied in this series is from 0.200 mg. to 0.750 mg. for 24 hours, averaging 0.420 mg. per 24 hours.

The serum antidiuretic activity (ADS) was estimated by the modification of the method of Birnie and his associates (31) described by Lloyd and Lobotsky (22). Antidiuretic activity is expressed in the following way: The percentages of a water load excreted at the end of a 90-minute period by rats that have been injected with

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the serum being assayed or with physiological saline solution are calculated. The difference between these percentages represents the antidiuretic activity. The antidiuretic activity is expressed as a negative value when the serum of the patients causes the rats used as test animals to retain water; whereas, it is expressed as a positive value when it causes the animals to excrete water. In a series of 39 determinations carried out on 5 normal men and 5 normal women an average antidiuretic activity of − 17 for each sex was found with a range of from − 41 to + 13.

Several specimens of urine from patients with toxemia have been subjected to chromatographic separation of the adrenal steroids by a modification of the method of Zaffaroni, Burton, and Keutmann (32) using a benzene-formamide solvent system on paper. By this method the major portion of the steroids excreted by non-pregnant individuals move at a rate characteristic of steroids containing 5 oxygens; a lesser amount of steroid has a rate of flow similar to those containing 4 oxygens.

PLAN OF STUDY

Seven normal pregnant women and eleven patients with pre-eclamptic toxemia were studied. The normal women collected 24-hour urine specimens at approximately two-week intervals. At the conclusion of the collection period a specimen of venous blood was drawn and the serum antidiuretic activity was estimated within ½ hour after the blood was taken. The measurements were carried out as frequently as possible on the toxemic patients.

RESULTS

Normal Pregnancy

The findings for normal pregnancy are summarized in Tables I and II. Pregnancy has been divided roughly into three trimesters. The last trimester has been considered as beginning at the

TABLE I

The serum antidiuretic activity, total corticosteroid, pregnanediol, and chorionic gonadotrophin levels in normal pregnancy

Type and number of subjects	Serum antidiuretic activity		Total corticosteroid *mg./24 hrs.*		Pregnanediol *mg./24 hrs.*		Chorionic gonadotrophin *r.u./24 hrs.*	
	Number of samples	Mean ±S.E.*	Number of samples	Mean ±S.E.	Number of samples	Mean ±S.E.	Number of samples	Mean ±S.E.
Non-pregnant (10 subjects)	39	−17±1.9	64 (31 subjects)	.420±.016				
1–13 Wks. pregnancy (3 subjects)	6	−21±5.5	7	.400±.052	10	12.2±2.2	13	10,300±1410
14–26 Wks. pregnancy (5 subjects)	18	−6±3.5	21	.496±.026	19	30.4±3.7	21	855±200
27th Wk.-delivery pregnancy (6 subjects)	15	−12±3.8	22	.600±.036	14	50.1±8.4	27	990±128

* S.E. = Standard Error.

TABLE II

Normal pregnancy—specimens on which benzene to water partitions of corticosteroids were performed

Type and number of subjects	Total corticosteroid *mg./24 hrs.*		Freely-water-soluble corticosteroid *mg./24 hrs.*		Poorly-water-soluble corticosteroid *mg./24 hrs.*	
	Number of samples	Mean ±S.E.	Number of samples	Mean ±S.E.	Number of samples	Mean ±S.E.
Non-pregnant (13 subjects)	22	.419±.029	22	.386±.028	22	.054±.016
1–13 Wks. pregnancy (3 subjects)	6	.393±.061	6	.330±.064	6	.070±.034
14–26 Wks. pregnancy (5 subjects)	20	.496±.026	20	.375±.024	20	.107±.015
27th Wk.-delivery pregnancy (6 subjects)	20	.633±.037	20	.472±.032	20	.161±.024

TABLE III

The serum antidiuretic activity, total corticosteroid, pregnanediol, and chorionic gonadotrophin levels in toxemia of pregnancy

Type and number of subjects	Serum antidiuretic activity		Total corticosteroid mg./24 hrs.		Pregnanediol mg./24 hrs.		Chorionic gonadotrophin r.u./24 hrs.	
	Number of samples	Mean ±S.E.	Number of samples	Mean ±S.E.	Number of samples	Mean ±S.E.	Number of samples	Mean ±S.E.
All cases of toxemia (11 subjects)	18	−15 ±3.9*	25	1.010±0.204†	19	34.5±5.9	39	5,130±850
Severe toxemia (6 subjects)	12	−17 ±4.9	10	1.635±1.27‡	14	34.8±7.3	27	6,320±1,190
Moderate and mild toxemia (5 subjects)	6	−10.5±5.5	15	.700±0.067	5	34 ±12.5	12	3,683±960

* The difference between this value and the average serum antidiuretic activity of all normal pregnancies (27th week to term) is not significant. $t = 1.7$, $p = .1$

† The difference between this value and the average total corticosteroid of all normal pregnancies (27th week to term) is not statistically significant. $t = 1.65$, $p = .1$

‡ The difference between this value and the average total corticosteroid of all normal pregnancies (27th week to term) is highly significant. $t = 3.5$, $p = .01$

TABLE IV

Toxemia cases in which benzene to water partitions of corticosteroids were performed

Type and number of subjects	Total corticosteroid mg./24 hrs.		Freely-water-soluble corticosteroid mg./24 hrs.		Poorly-water-soluble corticosteroid mg./24 hrs.	
	Number of samples	Mean ±S.E.	Number of samples	Mean ±S.E.	Number of samples	Mean ±S.E.
All cases of toxemia (11 subjects)	19	.795±.105	19	.530±.083	19	.253±.136*
Severe toxemia (6 subjects)	5	1.200±.109	5	.800±.26	5	.390±.170†
Moderate and mild toxemia (5 subjects)	14	.680±.068	14	.435±. 05	14	.210±.033

* The difference between this value and the average poorly-water-soluble corticosteroid of all normal pregnancies (27th week to term) is not significant. $t = 1.91$, $p = > .05 < 0.1$

† The difference between this value and the average poorly-water-soluble corticosteroid of all normal pregnancies (27th week to term) is probably significant. $t = 2.5$, $p = > .01 < .02$

twenty-seventh week to permit a closer comparison of the patients with toxemia, all of whom were in the twenty-seventh week of pregnancy or later.

Serum antidiuretic activity: There was no difference in antidiuretic activity of serum between any of the three trimesters of pregnancy and no difference between the pregnant and the nonpregnant woman.

Excretion of "total corticosteroid": Excretion of "total corticosteroid" increased during pregnancy, with an average during the last trimester which is near the upper limit of the normal range for nonpregnant women. During this trimester, many normal pregnant patients excreted amounts of corticosteroid considerably above the normal nonpregnant range.

Excretion of "freely-water-soluble" and "poorly-water-soluble corticosteroid": During the first trimester almost all the "total corticosteroid" was water soluble. During the second and third trimester, an appreciable amount of corticosteroid that did not pass from benzene into water was present. During the third trimester, approximately 25 per cent of the "total corticosteroid" was poorly-soluble in water.

Excretion of pregnanediol: An increase in pregnanediol excretion occurred throughout pregnancy, reaching a peak just before delivery.

Excretion of chorionic gonadotrophin: The urinary chorionic gonadotrophin level was highest during the first trimester, subsequently falling and

remaining at a low level throughout the rest of pregnancy.

Patients with Toxemia of Late Pregnancy

Eleven patients with pre-eclamptic toxemia were studied. All these patients were in the twenty-seventh week of pregnancy or later. These studies are summarized in Tables III and IV. A comparison of the values of normal pregnancy and of pre-eclamptic toxemia is shown in Figure 1.

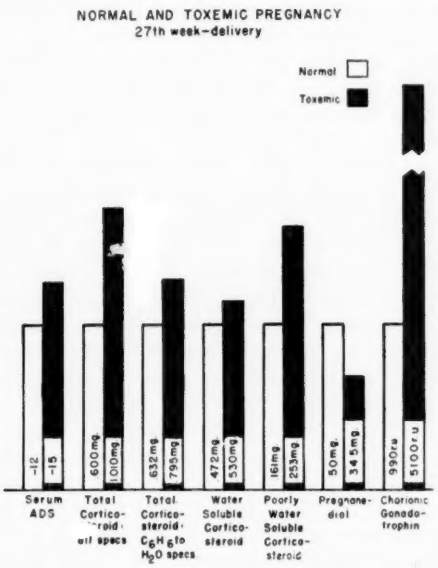

FIG. 1. SERUM ADS, URINARY CORTICOSTEROID, PREGNANEDIOL AND CHORIONIC GONADOTROPHIN IN SEVEN NORMAL AND ELEVEN TOXEMIC PREGNANCIES

Serum antidiuretic activity: The average of the 18 determinations was − 15. The range of these values was large and the difference between the activity of the patients with pre-eclamptic toxemia and the normal pregnant women was not statistically significant.

Excretion of "total corticosteroid": The twenty-five determinations on specimens from the 11 patients showed an average of 1.010 mg. of corticosteroid in 24 hours with a range of from 0.265 mg. to 4.750 mg. per 24 hours. This average was 61 per cent higher than the average for the third trimester of normal pregnancy.

Excretion of "freely-water-soluble" and "poorly-water-soluble corticosteroid": In addition to the "total corticosteroid" determination, benzene to water partitions were carried out on 19 specimens collected from patients with pre-eclamptic toxemia. The average "total corticosteroid" for these 19 specimens was 0.795 mg. per 24 hours. Of this corticosteroid 0.530 mg. was freely-water-soluble and 0.253 mg. was poorly-water-soluble.

Excretion of pregnanediol: The average value of pregnanediol for nineteen 24-hour specimens was 34.5 mg., which is considerably lower than the average for the normal subjects.

Excretion of chorionic gonadotrophin: A total of 39 measurements were carried out on specimens from the eleven patients with toxemia. The average excretion was 5,130 r.u. for 24 hours which was over five times higher than the average for normal pregnancy.

Comparison of mild and severe pre-eclampsia: The eleven patients with toxemia were divided into six "severe" cases, one "moderate" case, and four "mild" cases of pre-eclampsia. This purely arbitrary division of cases was made by the obstetrician (E. C. H.) without knowledge of the antidiuretic activity or corticosteroid values of the patients. Those patients with "severe" eclampsia had most of the following features: severe hypertension, severe edema, convulsions, albuminuria of 4 plus or greater degree, premature labor, and fetal death. The "moderate" or "mild" pre-eclamptics had moderate weight gain with slight edema, slight hypertension, and little albuminuria. The patients with more severe toxemia tended to have higher corticosteroid excretion, larger amounts of "poorly-water-soluble corticosteroid," and higher chorionic gonadotrophin excretion than are found in the patients with "moderate" or "mild" toxemia, although the number of determinations is insufficient to be statistically significant. The "moderate" or "mild" toxemia patients showed little, if any, deviation of the values from the normal range.

Fractionation by paper chromatography of urinary corticosteroid: Urines of two patients with pre-eclampsia have been studied by chromatography. In one subject, all the corticosteroid moved at a rate characteristic of steroids containing 4 or 5 atoms of oxygen. The major portion of the steroid moved at a rate characteristic of the 5-

oxygen-containing steroids. The other urine also contained a good deal of material that moved in a manner characteristic of 5- and 4-oxygen-containing steroids. There was, in addition, a considerable quantity of alpha-ketol which moved much more rapidly and had a rate of flow similar to that of desoxycorticosterone in a benzene-formamide system. When it was rechromatographed in a cyclohexane-formamide system it moved slightly more rapidly than desoxycorticosterone.

DISCUSSION

Is there an explanation for the discordance in results reported by those who have studied antidiuretic activity in pregnancy and toxemia? Several investigators have found increases in amounts of antidiuretic activity in normal pregnancy or in toxemia in the urine (11–13), but most of the workers who have looked for antidiuretic activity in serum have failed to find increased amounts of it (5–9). A possible explanation might be as follows: the antidiuretic activity of a material represents the algebraic sum of those factors which cause diuresis and of those which produce antidiuresis. Since adrenal cortical steroids are definitely diuretic in the rat, which is the usual test animal for antidiuretic activity, and since these steroids are considerably increased in toxemia and in normal pregnancy, it seems entirely possible that even if blood or plasma were to contain an increased amount of antidiuretic substance, the activity of this material might be masked by the increased amount of corticosteroid which would be injected into the rats when whole serum or plasma was given. On the other hand, if an antidiuretic material in urine were being sought, there would probably be no corticosteroid in the preparation given to the test animals because the technic of concentration of the urinary antidiuretic material is usually one which removes corticosteroids. For this reason, it is entirely possible that an increased amount of antidiuretic activity might be present in blood, but this activity might be balanced by an equally increased amount of diuretic substances, perhaps as a result of the body's compensatory mechanisms. And it is also possible that if there were increased amounts of antidiuretic activity, it could be detected in urine because the diuretic activity would not be measured.

The major objection to measurements carried out for urinary antidiuretic activity is that the test animals respond by profound antidiuresis to any noxious substance, and since the urine of women with pre-eclampsia is often extremely foul, containing albumin, bacteria, as well as possible unknown toxic products, it is well within the realm of probability that some of the antidiuretic activity of the urinary residues might be the result of response to a toxic substance.

The significance of the increased poorly-water-soluble material, which is found in the last trimester of pregnancy and which is further increased during toxemia, is at present unknown. Our evidence concerning the identity of this poorly-water-soluble fraction is not complete, but it can be said that if desoxycorticosterone were present in the urine, it would probably fall in this fraction. For example, when desoxycorticosterone was given in a large dose to a patient with adrenal insufficiency, the corticosteroid increase was entirely in this fraction (33). The formation by the body of the poorly-water-soluble material appears to be influenced by the administration of desoxycorticosterone. When a patient who was normally excreting an appreciable amount of poorly-water-soluble corticosteroid was given desoxycorticosterone, essentially no change in excretion occurred until the exogenous steroid was stopped, at which time the poorly-water-soluble material disappeared completely from the urine for several days (29). This suggests that the secretion of the poorly-water-soluble material was inhibited by the dose of desoxycorticosterone. It is not known whether the poorly-water-soluble material corresponds to the substance which moves on paper chromatography at a rate characteristic of 3-oxygen-containing steroids. However, a patient who had a significant amount of the material which moved on chromatography at a rate characteristic of a 3-oxygen-containing steroid also had an appreciable amount of poorly-water-soluble corticosteroid.

With full realization that identification of the poorly-water-soluble fraction and of the material which chromatographs in a manner characteristic of the steroids with 3 oxygen atoms is essential, and that any hypothesis must remain purely tentative until such identification has been made, we feel that the following question may justifiably be raised at this time. Is it possible that an increased

amount of desoxycorticosterone-like material may be of importance in the pathogenesis of eclampsia? If a material similar to desoxycorticosterone in its salt-retaining effects were present, it could easily explain the salt and water retention which is so characteristic of eclampsia. If its other properties were also similar to those of desoxycorticosterone, an excess of this steroid could produce many of the other aspects of eclampsia, especially the vascular changes which are so pathognomonic of this disease.

The increased corticosteroid excretion in eclampsia is due not only to the poorly-water-soluble material, but also to a considerable fraction of freely-water-soluble steroid. This freely-water-soluble fraction includes steroids with 5 oxygens such as cortisone and 17-hydroxycorticosterone. The increased levels of steroids with 5 oxygens might represent a compensatory mechanism, since in many respects these steroids can antagonize the effects of desoxycorticosterone (1). The antidiuretic material found in the urine of patients with eclampsia might be analogous to the increased antidiuretic activity which Skahen and Green (34) have found in the urine of rats chronically treated with DCA pellets and which is probably the result of stimulation of posterior pituitary secretion by the retained salt, or it might be the result of the deficient antidiuretic hormone inactivation by the damaged liver which is found in this disease (35). The increased level of 5-oxygen-containing steroids in the blood might prevent detection of any increased antidiuretic substance when serum is assayed. The vascular changes of the placenta could, of course, account for the altered excretion of chorionic gonadotrophin and pregnanediol which are seen in this disease (23, 26).

The normal pregnant woman also excretes an amount of poorly-water-soluble material which is greater than is found in the nonpregnant individual. However, this fraction of the corticosteroid is relatively smaller in the normal woman than it is in the toxemic. It seems possible that the relatively greater amount of freely-water-soluble corticosteroid proportionate to poorly-water-soluble corticosteroid found in the woman who does not develop toxemia, might reflect a normal compensation for the development of the poorly-water-soluble fraction. Thus, the inability to produce the relatively larger amount of freely-water-soluble material might be in some way connected with the development of eclampsia.

There is a small amount of other work which offers at least suggestive confirmation of this concept. Shipley (36) has found an increased amount of corticosteroid which has salt retaining activity when measured by bio-assay in the urine of patients with toxemia. Venning (37) has measured formaldehyde-forming corticosteroid and glycogen-depositing corticosteroid in women with toxemia, and has found that there is present a considerable amount of formaldehyde-forming material which does not cause glycogen deposition and that this material is less in amount in the normally pregnant individual. It is possible that in the human there may be a material analogous to the steroid recently isolated by Grundy, Simpson, and Tait (38) from beef adrenal which is highly active as a salt-retaining steroid.

Further proof of the validity of this concept would of course be afforded by the demonstration that administration of desoxycorticosterone can produce eclampsia and that administration of 5-oxygen-containing steroids would benefit patients with pre-eclampsia. Relatively little evidence is available on these points, but what is known is somewhat conflicting. Sobel (39) has given moderate amounts of DCA to normal pregnant women without effect. The dose was not large nor was it continued for long. Her work does not eliminate the possibility that a desoxycorticosterone-like substance is active in producing eclampsia, but it certainly does not add weight to this concept. Long-continued administration of relatively large doses of DCA would be necessary to furnish a definite answer to this question. It has been reported (40) that cortisone is definitely of value in severe eclampsia when other more usual methods of therapy have failed. On the other hand, Jailer (41) has found no benefit from the administration of ACTH to pre-eclamptic patients.

SUMMARY

1. No increase in antidiuretic activity of serum is detectable during normal or toxemic pregnancy.

2. Total corticosteroid increases throughout pregnancy, reaching a peak before delivery. Freely-water-soluble corticosteroid increases throughout pregnancy. A poorly-water-soluble

material appears during the first trimester and increases until the end of pregnancy, constituting 25 per cent of the corticosteroid.

3. In toxemia corticosteroid is considerably increased above the level found in normal pregnant women at the same stage of pregnancy. The poorly-water-soluble component is also considerably increased above that seen in the normal, representing approximately 35 per cent of the total corticosteroid. In one patient with eclampsia, paper chromatography demonstrated a material which has a rate of flow characteristic of 3-oxygen-containing steroids.

4. The possibility that this material might play a part in the etiology of eclampsia is discussed.

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AZORUBIN-BINDING CAPACITY AND PROTEIN COMPOSITION OF SERUM OF RATS SUBJECTED TO TOURNIQUET SHOCK AND TO TREATMENT WITH CARBON TETRACHLORIDE

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The cause of a decreased azorubin-binding capacity (ABC) of serum albumin, previously observed in humans under certain pathological conditions (1), is not known. The present paper is part of an experimental study into the mechanism of a lowering of the ABC (2). It describes the production of decreased ABC values of serum albumin of rats by subjecting them to tourniquet shock or to treatment with carbon tetrachloride.

In addition, the serum protein composition of these experimental rats was analyzed, since changes in the protein metabolism have been observed in traumatic shock (3, 3a).

EXPERIMENTAL

Chemical procedures. For the determination of the protein and non-protein nitrogen, the micro-Kjeldahl method of Hiller, Plazin, and van Slyke (4) was used, with some modifications which are described elsewhere (5). The non-protein nitrogen was separated from the serum proteins by precipitation with uranyl acetate according to the procedure of Neubauer (6). One ml. of serum was diluted with 3 ml. of water, and 1 ml. of 1.5 per cent uranyl acetate was added, with stirring, to the solution. The protein was filtered off after 60 minutes, using a Whatman No. 50 filter.

Since preliminary experiments indicated that a chemical determination of serum albumin with a 26.8 per cent sodium sulfate solution (7, 8) gave low values (for details see [5]), all albumin determinations were done by electrophoretic analysis.

Electrophoretic analysis. The veronal-acetate-sodium chloride buffer of Michaelis, pH 8.6, $\mu = 0.1$, was employed (9). Practically identical patterns were obtained in veronal buffer as used by Moore, Levin, and Leathem for rat sera (10). All pH measurements were done at room temperature with a glass electrode. The electrophoretic analyses were carried out in the compact Tiselius apparatus[1] (11), employing the Longsworth scanning mechanism and the 2 ml. cell in an open system. Some sera were also analysed in a standard size Tiselius electrophoresis apparatus[2] using the long analytical cell (12); practically the same patterns and the same albumin percentages were obtained. The albumin concentrations were defined according to Wiedemann (13) and determined from both the descending and ascending boundaries. The globulin area was divided, by the method of Tiselius and Kabat (14), into three parts corresponding approximately to the alpha-, beta-, and gamma-globulin of human sera. More details on the electrophoretic analysis are given elsewhere (5).

For the electrophoretic analysis of normal rat serum in the presence of azorubin, a solution of 5 mg. purified azorubin (for purification see [15]) in 8 ml. buffer was added to 4 ml. of serum and dialysed. The buffers used were Michaelis buffer pH 8.6 and phosphate buffers of pH 8.6, 7.7, 7.2, and 6.1, $\mu = 0.1$. The patterns were recorded on Kodak Ektachrome Color Film, using the optical system of Philpot-Svensson and a diagonal bar in the standard size electrophoresis apparatus.

Determination of ABC. The chromatographic method for estimating the ABC (16) was modified so that only 1 ml. of serum was needed for the single chromatographic runs. A total of 4 or 3 ml. of serum was required, therefore, for each analysis, which consisted of a blank run and triplicate, or at least duplicate, runs of the serum-azorubin mixtures (see below). The chromatographic tubes employed were about 20 cm. long, with an inner diameter of 5 mm., and terminated in a 0.5 mm. capillary of 2 to 3 cm. length. A funnel was sealed to the upper end. The columns were formed using a slurry of 500 mg. of anionotropic aluminum oxide in freshly prepared, twice-distilled water. Methods for preparation and deactivation of anionotropic aluminum oxide have been described previously (15, 16). The length of the column obtained was about 25 mm. The average total flow time for the serum-azorubin mixture described below was 9.5 minutes.

For each run, 1 ml. of serum and 0.25 ml. of an azorubin solution (mostly 0.5 per cent) in 0.6 per cent sodium chloride (pH 7.8) were combined at least 30 minutes prior to the analysis. The azorubin concentration in the mixture should be at least twice as high as the "observed ABC" (see below). For the blank run, 0.25 ml. of 0.6 per cent sodium chloride was added to 1 ml. of serum. The mixtures were poured on the column immediately after the water (used to wash in the aluminum oxide) ceased to run out of the capillary. The first 1.0 ml. of the filtrate was discarded and 2 dilutions (1:20) of the last 0.25 ml. (pH 6.1) were made with M/15 phosphate

[1] Model 38 of the Perkin-Elmer Corporation, Glenbrook, Conn.

[2] Frank Pearson Associates, New York 12, N. Y.

buffer, pH 7.7 (0.1 ml. + 1.9 ml. buffer). The optical density was determined in a Coleman Junior Model 6A spectrophotometer at 515 mμ using 10 × 75 mm. tubes. The azorubin concentrations were read in mg. per cent from standard curves prepared as described elsewhere (15). The value obtained is the "ABC observed." This modified procedure has been found (15) to give the same results as the previous method (16).

The "specific ABC" was calculated in moles azorubin per gram of albumin, according to

$$ABC_{spec.} = \frac{ABC_{obs.} \cdot 10^{-3}}{(0.8\,C - A)\,502}$$

where $ABC_{obs.}$ = ABC observed, in mg. per cent;

C = albumin concentration of serum, in grams per cent;

A = 0.13 for values of C > 2.9%;
0.12 for values of C < 2.9%.

The correction factor "A" accounts for the amount of albumin adsorbed by the aluminum oxide (see reference 15, Table 7). Studies on reproducibility and reliability of the chromatographic procedure for the estimation of the ABC have been described (15).

Animal experiments. Male Sprague-Dawley rats, fed on Purina Dog Chow checkers, were used. The average temperature of the animal room was about 24° C. The rats were left without food and water for 24 hours before the experiments. Weights given were those of the fasted animals. Tourniquet shock was produced as in previous studies (17). The rubber bands were applied to both hind limbs for 4½ hours and the blood was drawn by heart puncture 3 hours after release of the tourniquets. The average yield per rat in "shock" was about 2 ml. of blood and 0.5 ml. of serum, whereas 6 ml. of blood and 2.2 ml. of serum were obtained from the normal rats.

The carbon tetrachloride (C. P. Medicinal) was injected intraperitoneally (0.5 ml. per kg. every second day for from 7 to 16 days) (18). Some rats that did not lose weight after several injections were then given intraperitoneal injections of a 1:1 mixture of carbon tetrachloride and 95 per cent ethanol (1 ml./kg). The treatment with this solution (19) caused the weight of the animals to decline rapidly. The histological examination of the livers of a number of rats showed toxic degenerative changes which were consistent with the known picture of carbon tetrachloride poisoning. The amounts of blood and serum obtained from the carbon tetrachloride-treated rats were approximately the same as those from normal animals. Since the amount of serum obtained from one rat was not sufficient for the various analyses, pooled samples were used in all groups, as indicated in Table I.

RESULTS

The electrophoretic analysis of normal rat sera in the presence of azorubin demonstrated that this dye is bound exclusively to the albumin component, at pH values between 6.1 and 8.6.

TABLE I

Azorubin-binding capacity (ABC) of serum albumin of normal, shocked, and carbon tetrachloride-treated rats

1 Serum pooled from No. of rats	2 Average weight gm.	3 NPN mg. %	4 Albumin %	5 Globulin %	6 Specific ABC 10^{-6} moles azorubin per gm. albumin
A. *Normal Rats*					
6	396	38.3	4.06	2.25	2.38
4	411	37.8	3.74	3.07	2.66
4	408	30.0	3.97	2.85	2.24
3	389	35.3	3.29	3.23	2.45
4	280	33.3	4.33	2.33	2.20
4	255	38.0	4.07	2.65	2.96
Average Values*	357	35.5	3.91	2.73	2.48
B. *Rats Subjected to Tourniquet Shock*					
8	365	70.0	3.97	2.97	2.02
13	344	75.5	4.84	2.02	1.70
13	322	78.5	3.36	3.15	2.17
23	266	83.5	3.17	2.26	1.83
12	276	84.5	3.56	2.01	1.80
11†	346	138.0	4.08	2.89	1.33
Average Values*	313	78.4	3.78	2.48	1.91
C. *Rats Treated with Carbon Tetrachloride*					
4	317/248‡	41.8	3.16	3.03	1.40
4	318/264	43.5	3.09	2.58	1.91
5	314/223	46.5	3.07	2.64	2.04
4	292/251	43.0	3.69	2.48	1.72
4	310/241	49.0	3.01	2.27	1.85
5	307/270	48.8	2.88	1.99	1.27
4	345/305	43.8	3.84	2.87	1.43
Average Values*	315/257	45.2	3.25	2.55	1.66

* All average values were calculated as arithmetical means of the figures obtained, not considering the varying number of animals in the groups.

† Tourniquets applied unilaterally to the left fore and hind legs for 5 hours, blood drawn 17 hours after removal. Not included in average values.

‡ Weights before and after treatment.

The results of the serum analysis and the ABC values are given in Table I. The average specific ABC values of the serum albumin of the shocked and carbon tetrachloride-treated rats were 23 per cent and 33 per cent, respectively, lower than those of the normal controls. In one group of rats which were subjected to more severe conditions of tourniquet shock, a 46 per cent decrease of the ABC was observed.

Table II shows the percentage values of the electrophoretic protein components for the three series of rats.

TABLE II

Electrophoretic analysis of serum proteins of normal, shocked, and CCl_4-treated rats

Protein components in per cent of total protein

Rats, treatment	Total protein %	Albumin	Globulin		
			Alpha	Beta	Gamma
Normal	6.31	63.9	9.9	17.3	8.9
Normal	6.81	54.9	10.1	22.1	12.9
Normal	6.82	58.2	14.6	17.9	9.3
Normal	6.52	50.4	18.6	20.3	10.7
Normal	6.66	65.0	12.6	14.9	7.5
Normal	6.72	60.6	8.2	15.1	16.1
Average	6.64	58.9	12.3	17.9	10.9
Tourniquet Shock	6.94	57.2	8.7	23.5	10.6
Tourniquet Shock	6.97	58.5	16.1	19.3	6.1
Tourniquet Shock	6.86	70.6	8.2	17.0	4.2
Tourniquet Shock	6.51	51.6	10.2	23.0	15.2
Tourniquet Shock	5.43	58.4	13.0	19.2	9.4
Tourniquet Shock	5.57	63.8	11.4	16.2	8.6
Average	6.38	60.0	11.3	19.7	9.0
CCl_4-Treatment	6.19	51.1	22.1	19.7	7.1
CCl_4-Treatment	5.67	54.5	19.5	16.6	9.4
CCl_4-Treatment	5.71	53.8	24.9	15.3	6.0
CCl_4-Treatment	6.17	59.8	17.3	16.7	6.2
CCl_4-Treatment	5.28	56.9	14.8	19.2	9.1
CCl_4-Treatment	4.87	59.2	14.6	18.4	7.8
CCl_4-Treatment	6.71	57.3	16.0	18.4	8.3
Average	5.80	56.1	18.5	17.7	7.7

DISCUSSION

Analyzing the data of Table II, it should be pointed out that the electrophoretic analysis of the rat sera did not result in patterns in which the different globulin components were as distinct as generally seen with human sera. This is in accordance with observations of Gjessing and Chanutin (20). The separation of the globulin area into the three components is, therefore, rather arbitrary, even more than in the differentiation of the globulins in human sera (21).

In spite of this limitation, Table II shows that subjecting rats to tourniquet shock, under the conditions described, did not change markedly the concentration of albumin or of the globulin components in the serum. The results did not indicate any shift of serum albumin from the circulating blood into the injured tissues as demonstrated by a different experimental procedure by Moore and his associates in mice (22) and dogs (23) in traumatic shock. The electrophoretic patterns of the sera of "shocked" rats (Table II) did not reveal the appearance of increased quantities of a protein with a mobility similar to gamma-globulin as observed in mice under certain conditions of severe traumatic shock (22).

The average absolute and relative albumin values of the carbon tetrachloride treated rats were lower than those obtained in the normal animals. A slight increase in the average alpha-globulin value was obtained in the rats that received carbon tetrachloride. High values of this globulin component have been found under various pathological conditions in several species, among others in injured rats (20).

The specific ABC values were determined on the basis of the finding that azorubin is bound exclusively to albumin in rat serum. A similar result was obtained previously with human serum (1, 16). Assuming a molecular weight for rat serum albumin similar to that of human albumin, 1 mole of serum albumin of normal rats was found to bind 1.71 moles of azorubin; the values for "shocked" rats were 1.32 and 0.92 moles, depending on the severity of the shock produced. In the serum of carbon tetrachloride-treated rats, 1.14 moles of azorubin were bound per mole albumin. It may be pointed out in this connection that the average specific ABC value obtained with the chromatographic procedure in humans was 5.8 moles of azorubin per mole of albumin in normal persons, whereas values down to about 1 mole were observed in pathological cases (1).

The present studies demonstrate that it is possible to lower experimentally the ABC of serum albumin in rats. This decrease of the specific ABC values is considered to be typical of the albumin of the experimental rats, and not to be caused by a lower concentration of "normal" albumin in the treated animals. Previous studies on the chromatographic method have shown (16) that the specific ABC value of a given serum albumin is lowered by an extreme reduction of the albumin concentration of the test solution. However, an investigation into the quantitative side of this effect (see reference 15, Fig. 2) has shown that the specific ABC value of a 3.25 per cent solution of human albumin is less than 0.2 per cent lower than that of a 3.91 per cent solution of the same albumin. Assuming the binding of azorubin to rat serum albumin and that to human serum albumin to be essentially similar, a difference of about 0.2 per cent would be expected between the specific ABC values of the sera of normal and those of carbon tetrachloride-treated rats (albumin concentration 3.91 and 3.25 per cent, re-

spectively, see Table I), if such a difference were caused solely by the concentration effect described above. It seems improbable, therefore, that decreases in the specific ABC 100 to 200 times as great (23, 33 or 46 per cent) as observed between the normal and experimental groups (Table I) could be explained on the basis of differences in the albumin concentrations.

The degree of decrease in the specific ABC values of the "shocked" rats was proportional to the severity of their condition (Table I, last group of "shocked" rats compared to the other groups). Analyzing the single data of all groups of rats studied (Table I), no proportionality was found between albumin concentration and the degree of lowering of the specific ABC values. These observations are considered to be additional evidence for the conclusion that the albumin concentration does not play an essential role in the lowering of the specific ABC values obtained in the experimental animals. Disregarding the species difference, this is in contrast to the claim of Bennhold, Ott, and Kallee (24) that a decrease in the specific ABC values of human sera is caused exclusively by a reduced albumin concentration.

An analysis of the mechanism of a lowering of the ABC of serum albumin is believed to be of interest since a decrease in the ABC is considered to be but one example of the general phenomenon of a lowered capacity of serum albumin to bind anions. Decreased binding values of serum albumin for another anion, phenolsulfonephthalein, have been observed in cancer patients (25) and in pregnant women (26).

It is believed by the present authors that the decrease in the ABC of serum albumin, observed in different species under various conditions, is not caused by chemical differences in the albumin molecules, but rather by the binding of certain anions, *e.g.*, fatty acids, to the albumin. A firm binding of such anions would block the binding sites for azorubin and thus reduce the ABC. This conclusion is based on the following findings:

No proportionality was observed between the specific ABC values of the albumin fractions isolated by the cold alcohol fractionation procedure from sera giving normal and sera giving decreased specific ABC values. No chemical differences in the protein composition were observed either between the albumin fractions obtained from normal ABC-sera and those from low ABC-sera (1). The significance of these negative results is limited, however, since alterations during the preparation of the albumin fractions cannot be excluded.

Any degree of decrease in the specific ABC of serum albumin could be produced by addition of various amounts of certain fatty acids and other anions to albumin solutions and normal sera (2). This effect was exerted by very small amounts of anions, *e.g.*, one mole of myristic acid per mole of human albumin decreased its specific ABC by 26 per cent, and 5 moles of stearic acid per mole of albumin lowered the ABC value of a human serum by about 30 per cent. It may be noted that in all these experiments the albumin concentrations of the test solutions containing the anions were equal to those of the control solutions.

Experiments are in progress to find out whether the albumin present in low ABC-sera contains greater amounts of firmly bound anions (higher fatty acids?) than that present in normal sera. The results of these studies may aid in an understanding of the relationship between the decreased specific ABC values and the pathological conditions under which they occur.

SUMMARY

1. The protein composition of the serum of rats subjected to tourniquet shock was found not to be significantly different from that of normal rats. Administration of carbon tetrachloride caused a slight elevation of the serum alpha-globulin level, and some decrease in the average albumin concentration.

2. Electrophoretic studies demonstrated that in rat serum the anionic dye azorubin is bound exclusively to albumin.

3. The azorubin-binding capacity of serum albumin of rats was decreased in tourniquet shock and after treatment with carbon tetrachloride.

4. This lowering of the specific azorubin-binding capacity was not caused by and not related to a decrease in the albumin concentration.

5. A possible mechanism of a decrease in the azorubin-binding capacity of serum albumin is suggested.

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RENAL WATER EXCRETION IN PREMATURE INFANTS[1, 2]

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It is generally believed that, after water ingestion, the young infant can neither dilute his urine nor increase the rate of urine flow as rapidly or as effectively as the adult (1–4). Although several sound investigations (4–6) have demonstrated that the kidneys of newborn animals are relatively inefficient in this regard, there have been very few direct observations on these important functions in infants. The present investigation was undertaken, therefore, to compare the renal response of the young premature infant with that of the older infant and adult to ingestion of water.

PROCEDURE

Nine series of observations were made on a total of 8 normal subjects including one observation in each of 4 premature infants ranging in weight from 1.84 to 2.21 Kg. and in age from five to eight days, in one premature infant 36 days old and weighing 2.46 Kg., and in 2 adults, and 2 observations in one 18-month-old infant. The premature infants were all receiving half-skimmed cow's milk formulas. All observations were made in an air-conditioned metabolism unit in which the temperature and humidity were maintained at 23° C. and 50 per cent respectively and the subjects were lightly clothed to prevent sweating. The technics used for collecting blood and urine samples in premature infants have been described (7) and the same catheterization-infusion technic was used in the older infant and adults. Because it was observed that in premature infants venipunctures affected the functions being investigated (see Comment) the following plan was followed: three to six hours after the infant's last feedings and after 12 hours of fasting and thirsting in the adults, an initial blood sample was drawn, the bladder catheter was inserted, and a pre-hydration urine-collection period was started. At this same time a polyethylene nasal tube was passed into the stomachs of the premature infants and of the 18-month-old infant. The presence of the tube was well tolerated. After 1 to 1½ hours, the infants were given water via the polyethylene tube and the adults drank water in doses equal to 40 ml. per Kg. for the premature infants, 30 ml. per Kg. for the 18-month infant, and 20 ml. per Kg. for the adults. Per M^2, *these doses of water were equivalent to 507 to 530 ml. in the infants, 640 ml. in the 18-month-old infant, and 639 and 734 ml. in the adults.* Except for collecting urine samples, the subjects were left undisturbed for another 1 to 1½ hours, at which time a second blood sample was drawn.

The following measurements and calculations were made:

1. *Glomerular filtration rate* (GFR): estimated by creatinine clearances (C_{Cr}) calculated from creatinine concentrations of serum and urine measured by the method of Hare (8).

2. *Concentration of osmotically active urinary solutes* (U_s): estimated by summating the milliosmolar concentrations of sodium (9), chloride (10), potassium (9), urea (11), phosphate (12) and creatinine (8). The sum of these values equalled 89 to 96 per cent of the total milliosmolar concentrations estimated from depression of the freezing points.

3. Rate of excretion of urinary solutes (U_sV): calculated as the product of U_s and urine flow (V).

RESULTS

The effect of water ingestion on urine flow (V) in the 4 young and one older premature infant and in the 2 adults is shown in Figure 1. A marked diuresis which began at approximately the same time after water ingestion was observed in all of the subjects. Corrected on the basis of surface area,[4] however, the increase in V in the young premature infants was only about one-half as great as in the older premature infant and in the adults.

Changes in the concentration of solutes in the urine (U_S) after water ingestion are shown in Figure 2. No difference was observed in the

[1] Presented in part before the American Pediatric Society at Old Point Comfort, Va., May 8, 1952.

[2] Supported in part by a research grant from The Playtex Park Research Institute.

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[4] Surface area of the premature infants was calculated by the formula $5.188 \times Wt.^{0.75}$ (13). Surface area of the older infant and adults was estimated from the nomogram of DuBois (14).

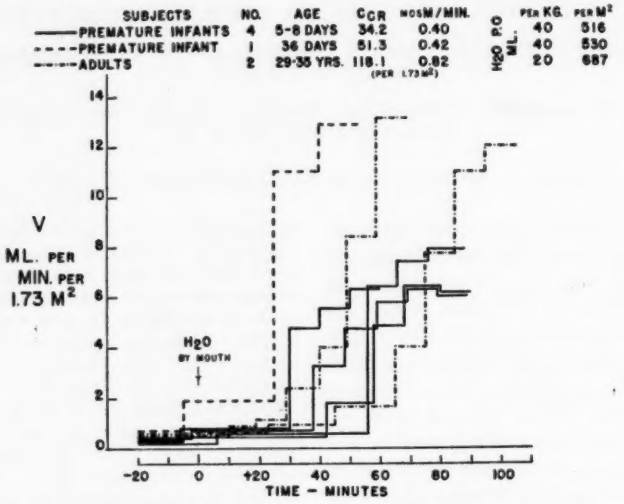
SUBJECTS NO. AGE C_{CR} mosM/MIN. H2O P.O ML. PER KG. PER M²
PREMATURE INFANTS 4 5-8 DAYS 34.2 0.40 40 516
PREMATURE INFANT 1 36 DAYS 51.3 0.42 40 530
ADULTS 2 29-35 YRS. 118.1 0.82 20 687
(PER 1.73M²)
V
ML. PER MIN. PER 1.73 M²
H2O BY MOUTH
TIME - MINUTES

FIG. 1. EFFECT OF WATER INGESTION ON RATE OF URINE FLOW (V)

rapidity with which or the extent to which the urine became diluted. Thus the lowest values for U_B in the 5 premature infants ranged from 40.6 to 63.0 mosM per L (average 55.8) compared with values of 64.8 and 47.7 in the two adults and of 48.1 and 39.0 in two observations in the 18-month old infant.

DISCUSSION

Under the conditions of these observations, it would appear that the premature infant responds

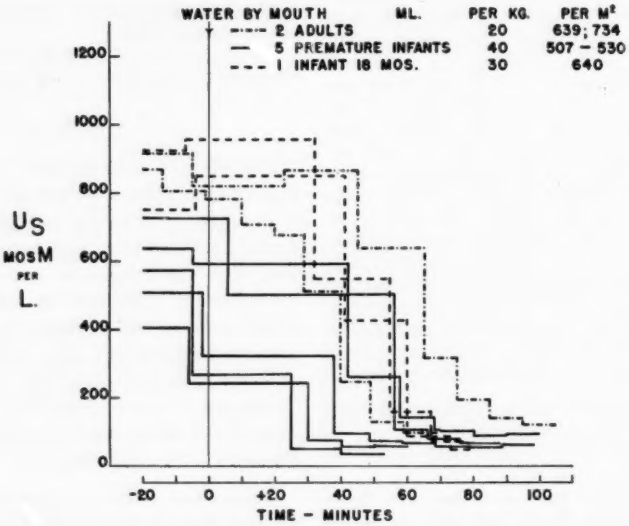
WATER BY MOUTH ML. PER KG. PER M²
2 ADULTS 20 639; 734
5 PREMATURE INFANTS 40 507 - 530
1 INFANT 18 MOS. 30 640
Us
MOSM PER L.
TIME - MINUTES

FIG. 2. EFFECT OF WATER INGESTION ON CONCENTRATIONS OF OSMOTICALLY ACTIVE URINARY SOLUTES (U_B)

to water ingestion by diluting the urine to the same minimum milliosmolar concentration as the adult, but that per 1.73 M^2 the infant attains a lower maximum rate of urine flow than the adult. This observation can be explained by at least two additional differences between the two groups of subjects. First, as shown in Figure 3, the glomerular filtration rate of water (GFR) in the premature infant was, on the basis of surface area, less than one-half that of the adult. Inasmuch as the maximum V per 1.73 M^2 in the premature infant was somewhat more than one-half that in the adult, it follows that a larger fraction of filtered water (V/C_{CR}) was excreted by the infant. In this sense, therefore, water diuresis was relatively more pronounced in the infant. Second, with GFR remaining constant, the degree to which V can be increased in the absence of antidiuretic hormone (ADH) is determined by the rate of solute excretion (U_SV). As shown in Figure 3, U_SV per 1.73 M^2 was only about one-half as high in the premature infant as in the adult. For the premature infant to have attained a maximum V equal to that of the adult a greater dilution of the urine would have been required; this may not have been possible, even after larger water loads. Thus far, we have not achieved conditions in which the premature infant and the adult have, on the basis of surface area, equal rates of solute excretion which would permit exclusion of this variable in comparing their response to water ingestion.

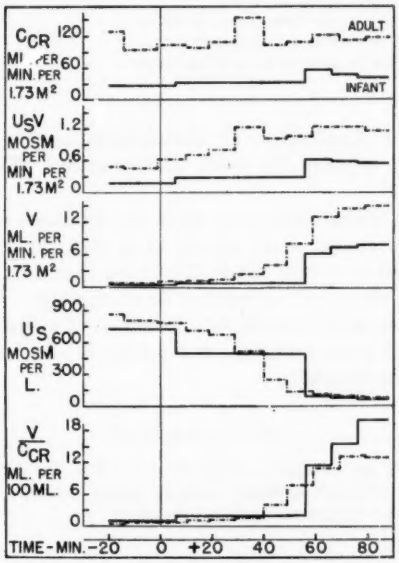

FIG. 3. EFFECT OF WATER INGESTION ON CLEARANCE OF CREATININE (C_{CR}), RATE OF SOLUTE EXCRETION (U_SV), RATE OF URINE FLOW (V) CONCENTRATION OF URINARY SOLUTES (U_S) AND PER CENT OF FILTERED WATER EXCRETED (V/C_{CR}) IN A PREMATURE INFANT AND IN AN ADULT

Water was given at 0 time in doses equal to 20 ml. per Kg. (639 ml. per M^2) for the adult and 40 ml. per Kg. (527 ml. per M^2) for the infant.

COMMENT

The results presented here are at least in part contrary to the earlier observations of Aschenheim (15) and of Lasch (16) and to the general belief that the young infant is limited in his ability to dilute his urine and to increase V promptly following water ingestion. Any one or combination of several possibilities may account for the failure of earlier investigators to observe similar responses to water ingestion. A lower maximum V would possibly have been observed in the infants if the dose of water had been given on the basis of weight rather than of surface area. However, since comparisons between rates of urine flow and other kidney functions are almost always made on the basis of surface area (3) it seems reasonable that the dose should be calculated on the same basis. Secondly, if the infants observed here had been receiving breast milk rather than cow's milk, the rates of solute excretion would have been still lower; this would have further limited the increase in V. Finally, and perhaps most important, painful stimuli such as venipunctures may have a profound effect on V in the premature infant (17). In the observation shown in Figure 4, diuresis following water by mouth was interrupted for almost an hour as the result of a venipuncture. This type of response to pain has been observed repeatedly and suggests that in earlier observations (15, 16) inadvertent disturbances of the infant may have been responsible in part for failure to observe an increase in V following water by mouth. It is of special interest that the fall in V following venipuncture was accompanied, not by an increase in U_S, but by a marked reduction in GFR. Decreases in V which followed injections of pitressin, given without disturbing the infant through the

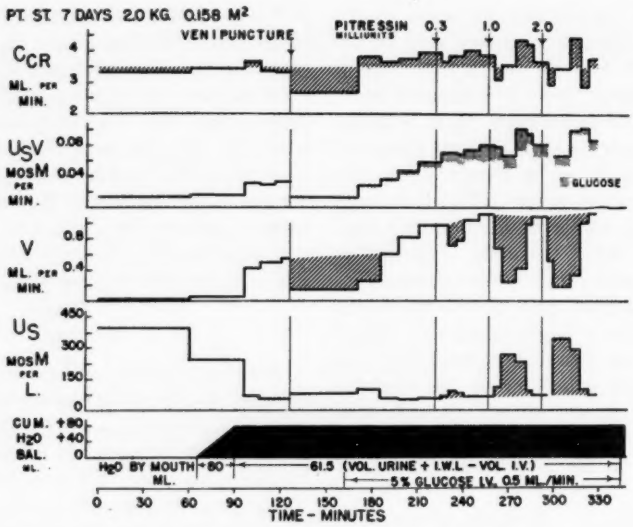

FIG. 4. EFFECT OF VENIPUNCTURE AND INTRAVENOUS INJECTIONS OF GRADED DOSES OF PITRESSIN ON CLEARANCE OF CREATININE (C_{CR}), RATE OF SOLUTE EXCRETION (U_SV), RATE OF URINE FLOW (V), AND CONCENTRATION OF URINARY SOLUTES (U_S) FOLLOWING WATER INGESTION IN A PREMATURE INFANT

tubing of a continuous intravenous infusion begun earlier, were, as expected, associated with a rise in concentration of urinary solutes and no real change in GFR.

CONCLUSIONS

The data presented here indicate that in comparison with older infants and adults, the young premature infant shows no limitation in his ability to dilute his urine following water ingestion. In the infant, maximum dilution of the urine occurs at relatively lower maximum rates of urine flow; this may be due to the fact that infants have, on the basis of surface area, lower rates of glomerular filtration and of solute excretion. Whether or not premature infants would show a similar response to water ingestion in the immediate postnatal period cannot be determined from these observations since the youngest premature infants ranged in age from five to eight days. The fact that a 36-day-old premature infant showed a response to water ingestion comparable in all respects to that of the adult, suggests a very rapid postnatal development of this function. A similar rapid development of concentrating ability has been reported by Pratt, Bienvenu, and Whyte (18).

A clinical implication which may be drawn from these results would appear to be that, per milliosmol of urinary solute, the young premature infant can excrete as much as 20 ml. of water. This value, which constitutes a definition of maximum renal water excretion, is the same for the infant as for the adult.

ACKNOWLEDGMENTS

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THE EFFECT OF INTRAVENOUSLY ADMINISTERED 6063, THE CARBONIC ANHYDRASE INHIBITOR, 2-ACETYLAMINO-1,3,4-THIADIAZOLE-5-SULFONAMIDE, ON FLUID AND ELECTROLYTES IN NORMAL SUBJECTS AND PATIENTS WITH CONGESTIVE HEART FAILURE

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Many of the clinical features of congestive heart failure are determined by the abnormal accumulation of water in the extracellular space, an event largely dependent upon the abnormal renal retention of sodium. Although there is a lack of knowledge of the exact mechanism responsible for this abnormality in renal excretion, it is well known that agents that enhance the excretion of sodium are valuable in the treatment of heart failure.

The normal kidney plays an important part in preventing the sodium depletion of body fluids and maintaining the normal range of pH of the blood. This is accomplished by the tubular reabsorption of almost all the sodium which is filtered through the glomerulus and the conversion of the alkaline glomerular filtrate into an acid urine (1). The enzyme, carbonic anhydrase, found to be present in the renal cortex as well as other tissues such as gastric mucosa and erythrocytes of mammals (2), is regarded as an important element in the mechanism for the acidification of the urine (3) and the reabsorption of sodium. The inhibition of carbonic anhydrase and the prevention of the formation of an acid urine may serve as a method for reducing the abnormal sodium retention which is a feature of congestive heart failure.

Following the observation that sulfanilamide induced acidosis (4) with an alkaline urine (5) and an increased excretion of sodium and potassium (6, 7) and the demonstration that sulfonamide compounds were specific carbonic anhydrase inhibitors (8), Höber suggested that the alkalinization of the urine was due to the inhibition of carbonic anhydrase in the renal tubules (9). Subsequently, Pitts and Alexander showed that sulfanilamide reduced the capacity of the kidney to eliminate acid under maximal stimulation by a phosphate buffer load (3).

Schwartz administered sulfanilamide to three patients with congestive heart failure and observed an increase in sodium, potassium, and water excretion (10). However, this drug was regarded as possessing too low an order of carbonic anhydrase inhibitor activity and as being too toxic for continued use.

Recently a series of more active carbonic anhydrase inhibitors of the sulfonamide group has been developed (11). One of these, the heterocyclic sulfonamide, 6063 (2-acetylamino-1,3,4-thiadiazole-5-sulfonamide), is a carbonic anhydrase inhibitor which is 50 to 400 times more effective than sulfanilamide and nontoxic in effective doses (12). Studies in dogs (13) and a preliminary report of studies in human beings (14) have indicated that this compound markedly increases sodium, potassium, and bicarbonate excretion. This is a report of the study of the urinary excretion of water and electrolytes and of the blood electrolyte pattern following the administration of 6063 [2] to hospitalized patients with and without congestive heart failure.

MATERIAL AND METHODS

The character of the clinical material is presented in Table I. Of the fifteen subjects, there were ten men and five women whose ages ranged from twenty-three to eighty-three years. Three of the subjects had no evidence of cardiovascular or renal disease and were used as controls. The remaining twelve were patients with congestive heart failure of varied etiology as listed in Table I. These patients had dyspnea on exertion, orthopnea, a prolonged circulation time and an increased venous pressure. All the cardiac patients had been treated in the previous few weeks and had little or no clinical evidence of edema at the time 6063 was administered. The one exception was patient J. K., who presented moderate edema of the lower extremities. Two of the control and four of the cardiac subjects were on

[1] Fellow of the Dazian Foundation.

[2] Supplied through the courtesy of Dr. J. M. Ruegsegger of Lederle Laboratories.

TABLE I

Patient	Age	Sex	Diagnosis	Sodium intake	Urine flow		24-Hour weight loss	Excretion rate										Urine pH	
								Na μEq./min.		K μEq./min.		Cl μEq./min.		HCO_3 μEq./min.		PO_4 μEq./min.		NH_3 μEq./min.			
					Cont.	Max.		Cont.	Max.	Cont.	Max.	Cont.	Max.	Cont.	Max.	Cont.	Max.	Cont.	Min.	Cont.	Max.
				gm.	cc./min.		Kg.														
S. E.	55	M	Control	5.0–7.0	3.3	4.3	1.9	75.8	307	51.6	150	105	164	—	—	45.1	67.9	6.3	0.9	(5.5)	(7.8)
J. C.	23	M	Control	5.0–7.0	4.3	7.6	0.5	92.0	545	44.8	246	158	328	—	—	54.8	108	22.8	2.5	6.21	7.62
R. B.	35	F	Control	0.5	2.7	6.3	0.5	17.4	178	18.7	128	47.6	170	2.1	146	36.0	82.0	10.5	7.3	6.00	7.40
M. G.	44	M	Beriberi	5.0–7.0	0.9	1.7	0.9	169	325	73.0	141	—	—	—	—	45.2	77.0	—	—	(5.0)	(7.7)
F. L.	57	M	A.S.H.D.	5.0–7.0	1.8	6.0	2.7	96.3	294	62.8	138	87.3	142	—	—	49.4	62.4	—	3.8	—	7.70
J. K.	42	M	Cor Pulmonal	5.0–7.0	6.7	20.5	1.1	139	920	82.9	214	117	201	66.3	812	65.5	149	17.6	4.9	6.63	7.53
I. Q.	65	M	A.S.H.D.	5.0–7.0	4.5	7.9	1.1	72.2	321	72.7	170	76.2	113	16.2	302	21.3	105	7.9	2.8	6.55	7.50
H. C.	83	M	A.S.H.D.	0.5	2.5	6.6	0.7	20.7	132	28.2	142	15.0	30.0	—	—	26.7	77.2	2.5	1.4	(6.5)	(7.8)
G. B.	56	M	R.H.D.	0.5	0.3	1.7	1.1	7.6	155	14.5	114	15.8	49.3	—	—	30.7	76.5	3.4	1.3	(5.0)	(7.8)
R. H.	39	M	R.H.D.	0.2	0.5	3.6	0.9	2.3	104	35.3	168	22.0	62.1	—	—	5.9	51.0	5.7	1.5	(5.0)	(7.8)
L. I.	37	M	R.H.D.	0.2	0.6	9.2	1.0	1.8	275	21.0	348	6.1	59.6	—	—	35.6	155	13.6	3.2	5.75	7.59
S. R.	47	F	H.C.V.D.	0.2	1.1	3.6	0.7	2.5	50.0	42.8	290	3.1	8.5	11.8	269	23.5	58.4	20.7	1.1	7.04	7.66
S. S.	50	F	R.H.D.	0.2	0.5	2.9	1.5	26.8	196	28.5	144	19.0	31.4	4.6	224	29.0	58.8	6.5	3.2	6.62	7.58
M. B.	28	F	R.H.D.	0.2	0.4	1.6	0.6	0.3	7.5	17.4	67.0	0.7	9.4	0.1	43.3	30.7	47.0	14.8	1.9	5.40	7.59
S. F.	58	F	A.S.H.D.	0.2	3.0	3.6	1.5	7.9	79.2	61.9	122	11.5	30.6	21.6	115	28.2	30.6	7.5	1.5	6.91	7.62

Sodium intake refers to average daily sodium of diet on days preceding the experiment.

Cont.—Average of the rates observed during the control periods.

Max.—Maximum rate observed after 6063. Min.—Minimum rate observed after 6063.

pH values in ( ) determined by indicators. All other pH values determined with glass electrode.

R.H.D.—Rheumatic Heart Disease.

A.S.H.D.—Arteriosclerotic Heart Disease.

H.C.V.D.—Hypertensive Cardiovascular Disease.

a diet containing 5.0 to 7.0 grams of sodium per day prior to the experiment. The remaining subjects were on diets restricted to 0.2 to 0.5 grams of sodium per day for at least three days prior to the day of the experiment (Table I). These differences in dietary intake in the various control and cardiac subjects were not related to the character or severity of clinical manifestations but were arbitrarily determined for the purpose of this study. All subjects were fasting for at least twelve hours prior to the onset of the experiment and received no food during the observation period, which averaged six hours.

Hydration was instituted three to four hours prior to the start of the infusion of 6063 and maintained until the end of the experiment. Except for patients J. K. and S. F., all subjects received 250 cc. of water orally per hour. J. K. received 1,000 cc. of water per hour for four hours prior to the beginning of the control period and 500 cc. of water by mouth per hour until the observations were discontinued. S. F. received 250 cc. per hour for three hours before the control period and during the entire experiment plus an additional 250 cc. at the beginning of the control period and at the start of the infusion of 6063. The volume of fluid given intravenously was included in determining the fluid intake. After a series of control periods, 750 mg. of 6063 in 250 cc. of 5 per cent dextrose in water was administered intravenously over a period of twenty minutes. However, patient M. B. weighed only 42.3 Kg. and received 400 mg. of 6063.

The urine from all the women and three of the men was obtained through an indwelling catheter; the other male subjects voided directly into a container from the standing position. Massage over the suprapubic region was carried out at the end of each collection period to promote emptying of the bladder. In the seven patients in whom the carbon dioxide content of the urine was determined the specimen was passed directly into a vessel containing mineral oil and analyzed promptly. Blood specimens were obtained from a vein and heparin was used as an anticoagulant. The blood specimens for pH and carbon dioxide content were obtained by venipuncture without stasis and drawn into a greased syringe containing heparin and sodium fluoride to prevent glycolysis.

Plasma and urine sodium and potassium determinations were performed on a Janke flame photometer with lithium as an internal standard. Chlorides were determined by the method of Schales and Schales (15), creatinine by the method of Bonsnes and Taussky (16), and phosphorus by the method of Fiske and Subbarow (17). Titratable acidity was titrated with 0.1 N sodium hydroxide, phenol red being used as the indicator. Ammonia content of the urine was obtained by the aeration method of Van Slyke and Cullen (18) as modified by Summerson (19). The pH of the blood was determined at room temperature with a glass electrode and a Cambridge pH meter and converted to body temperature by subtracting 0.01 unit for each degree below 38° C. The carbon dioxide content of blood and urine was determined manometrically by the method of Van Slyke and Neill (20). The bicarbonate content was calculated by the Henderson-Hasselbalch equation with 6.10 as the pK for both blood and urine.

RESULTS

Effect on urinary flow and body weight (Table I)

The urinary flow increased in each case and there was a consistent loss of body weight varying from 0.5 to 2.7 Kg. within twenty-four hours after the administration of the drug. The patients used were almost all free of edema as a result of previous therapy. Since 6063 was being used primarily as a physiological tool to investigate renal behavior in heart failure and not to demonstrate its efficacy as a diuretic, it did not appear important to choose subjects with considerable edema. These facts must be considered in evaluating the relatively moderate increase in urinary flow and loss of weight.

Acid-base factors (Figure 1)

Following the administration of 6063 the urine invariably became alkaline in thirty minutes and remained so for the duration of the observation period of about six hours. The highest urinary pH appeared after one to two hours, generally reaching a slightly higher level than that of plasma and varying between 7.40 and 7.70. Titratable

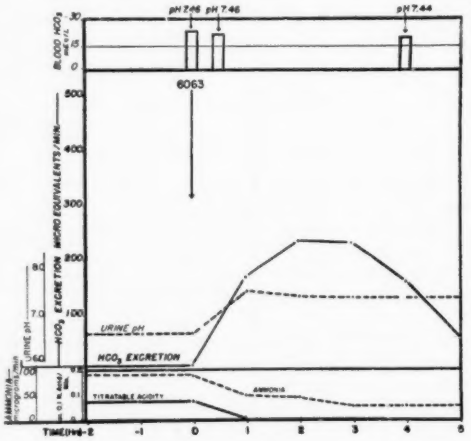

FIG. 1. THE EFFECT OF 6063 ON THE ACID-BASE REGULATORY ACTIVITY OF THE KIDNEY IN A PATIENT WITH CONGESTIVE HEART FAILURE AND ON A DIET RESTRICTED TO 200 MG. OF SODIUM DAILY

pH values at top of figure are those for blood.

acidity rapidly disappeared. The excretion of urinary ammonia was markedly diminished in all cases, but the ammonia continued to be present even when the urine was more alkaline than plasma.

Control specimens of urine were acid except in one patient, S. R., who was on a rice diet, and whose control urine had a pH of 7.04. In the control urines the bicarbonate excretion rate was low, ranging from 0.1 to 21.6 microequivalents per minute. In one patient, J. K., with cor pulmonale, the control bicarbonate excretion rate was 66.3 microequivalents per minute. In all cases there was a marked increase in bicarbonate excretion following the intravenous administration of 6063, the peaks ranging from 43 to 812 microequivalents per minute.

Blood bicarbonate and pH levels disclosed only a slight tendency to acidosis following a single intravenous administration of 6063. However, this evidence of acidosis due to 6063 was much more striking in other patients studied who were given 6063 orally for several days. In one such patient, R. B., who had received 600 mg. of 6063 orally per day for two days, the blood bicarbonate concentration fell to 18 milliequivalents per liter and was further reduced to 15 milliequivalents per liter after an intravenous injection of 6063. Despite the low blood bicarbonate concentration and a blood pH of 7.26 the urine was alkaline, containing a high concentration of bicarbonate and a low concentration of ammonia. The peak rate of bicarbonate excretion was 146 microequivalents per minute.

Effect on urinary electrolytes

The effect of intravenous 6063 on the individual major electrolytes in the urine is represented in Figure 2. These are the findings in S. S., a patient with congestive heart failure, who had been on a 500 mg. dietary intake of sodium. It is apparent that as the urinary flow increased there was marked increase in quantity as well as an increase in concentration of sodium, potassium, and bicarbonate, whereas chloride and phosphate excretion increased only slightly and less than the increase in flow.

The *sodium excretion* increased markedly in every instance, independently of the presence or absence of congestive heart failure. In the two control subjects receiving 5.0 to 7.0 grams of sodium daily, the sodium excretion increased to peak levels of between four and six times the control values. In absolute terms this represented an increase of 231 and 453 microequivalents of sodium per minute, respectively. In the normal subject receiving 500 mg. of sodium daily there was a tenfold increase in the rate of sodium excretion, but the absolute increase was only 161 microequivalents per minute. In the patients with congestive heart failure, the increment in sodium excretion appeared to be related to the sodium intake, being greater in those patients who were previously on a more liberal sodium diet. When the sodium was limited to 200 or 500 mg. daily, the absolute increase in sodium excretion varied from 7.2 to 273 microequivalents per minute. The differences between the increments of sodium excretion could not be correlated with any differences in the degree or type of heart failure or with any difference in responsiveness to therapy. There were insufficient data to determine whether there was a significant difference in the increments of sodium excretion between the control and cardiac groups of patients.

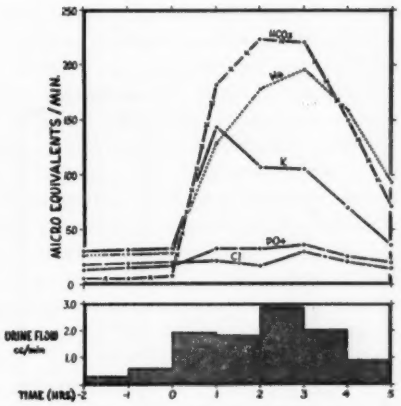

FIG. 2. THE EFFECT OF 6063 ON EXCRETION RATES OF WATER AND ELECTROLYTES

6063 was given at "0" time.

The *potassium excretion,* like that of sodium, increased in all cases after the intravenous administration of 6063. In general the degree of increase in potassium excretion did not vary as much as the increase in sodium excretion. The increment in potassium excretion bore no constant relationship to the increase in sodium excretion.

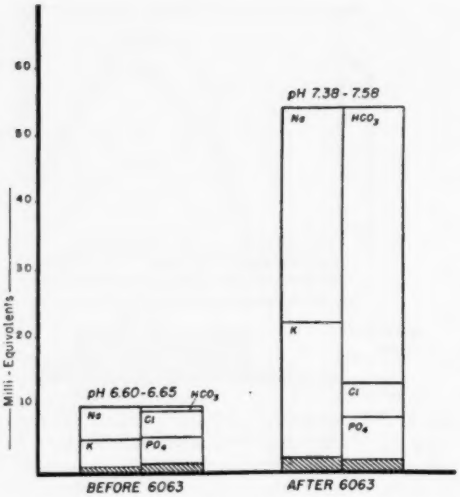

FIG. 3. THE TOTAL ELECTROLYTE CONTENT OF THE URINE FOR A THREE-HOUR PERIOD BEFORE AND A THREE-HOUR PERIOD AFTER THE ADMINISTRATION OF 6063

The cross-hatched areas are inserted as a diagrammatic representation of other unmeasured cations and anions but have no quantitative significance.

Chloride excretion was invariably augmented, but the absolute increase was quite small compared with that noted for sodium, potassium, and bicarbonate. Unlike the concentration of the latter electrolytes, that of urinary chloride was less following 6063 than preceding the administration of the drug. The absolute increase in chloride excretion appeared to be related to the increased urinary flow. The *excretion of phosphates* was increased consistently to the same or to a slightly higher degree than that of chloride.

The effect of 6063 upon the electrolyte spectrum of the urine and the change in total electrolyte excretion is represented schematically in Figure 3. The numbers denote the total excretion of electrolytes, in milliequivalents, for three hour periods before and after the intravenous administration of the drug. There are readily apparent absolute increases in potassium, sodium, and bicarbonate with relatively small increases in phosphate and chloride. In the case depicted, the single intravenous injection of 6063 increased the total excretion of base for a three-hour period by 45 milliequivalents.

Comparison of the effect of 6063 with that of a mercurial diuretic on urinary electrolytes

The difference between the pattern of urinary electrolytes after a mercurial diuretic and that after 6063 is indicated by the following representative observations on two patients, one of whom received an intravenous injection of 2 cc. of mercuhydrin and the other a single intravenous injection of 750 mg. of 6063. Both patients were essentially similar in respect to the type and severity of congestive heart failure and an almost identical diuresis and weight loss were observed in each. After the administration of 6063, the urinary flow rose from 0.6 to 9.2 cc. per minute, whereas after the mercurial the urinary flow increased from 0.2 to 11.4 cc. per minute. Both the onset and peak of action occurred earlier after 6063 than after the mercurial. Peak excretion rates of 275 microequivalents per minute for sodium and 59.6 microequivalents per minute for chloride were observed after the administration of 6063; maximum excretion rates after mercurial were 1,037 microequivalents per minute for sodium and 1,058 microequivalents per minute for chloride. Bicarbonate increased markedly after 6063, slightly after the mercurial.

Plasma electrolyte pattern

Reference has already been made to the observation that the relatively small effective single intravenous dose of 6063 administered in these experiments produced a slight and inconstant tendency towards acidosis as indicated by a reduction in the blood bicarbonate level and blood pH. No significant effect was noted on the plasma concentrations of sodium, potassium, chloride, phosphate, or creatinine.

Toxicity

No toxic effects were observed. The slight chemical acidosis that occurred in some subjects was not accompanied by any clinical symptoms.

DISCUSSION

The observations described above of the effect of the carbonic anhydrase inhibitor, 6063, on electrolyte excretion support the concept of Pitts and Alexander (3) that the enzyme, carbonic anhydrase, in the renal tubules plays an important

part in the acidification of the urine and the conservation of base. According to this theory, carbonic anhydrase accelerates the hydration of carbon dioxide to carbonic acid in the tubule cells and this acid, in turn, provides hydrogen ions which are exchanged for sodium ions in the tubular urine (Figure 4). The hydrogen ions combine with bicarbonate ions in the tubular lumen to form carbonic acid. This breaks down to provide carbon dioxide, which diffuses freely across the tubular membrane and into the tubular cell. The exchange of hydrogen for sodium ions converts the urinary alkaline phosphate to the acid form. The net result is the reabsorption of sodium and bicarbonate and the addition of hydrogen ions to the tubular urine. This process has generally been regarded as limited to the distal tubules (3), which are said to receive approximately the 15 per cent of glomerular filtrate not already reabsorbed in the proximal tubules (21). When, as in these experiments, the carbonic anhydrase was inhibited by 6063, both the sodium and the bicarbonate, which would have been reabsorbed if hydrogen ion were secreted, were now excreted and the urine remained alkaline.

It is of interest that the inhibition of carbonic anhydrase augments the excretion of sodium even from the kidney of a person in heart failure, despite the known tendency in such a kidney to increased reabsorption of sodium. The increment

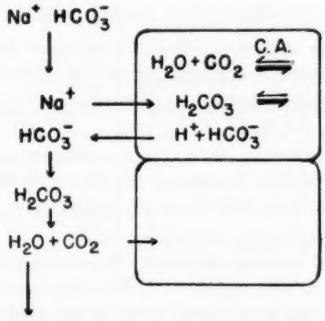

FIG. 4. MECHANISM OF HYDROGEN-SODIUM EXCHANGE AND OF BICARBONATE REABSORPTION

Carbonic anhydrase (C.A.) catalyses the hydration of CO_2 to carbonic acid which is a source of hydrogen ions. Sodium from the tubular urine is exchanged for this hydrogen ion. In the lumen this hydrogen ion combines with bicarbonate to form carbonic acid and subsequently H_2O and CO_2. The CO_2 diffuses back into the tubule cell.

in sodium excretion following 6063 is limited to that portion of filtered sodium which depends on the secretion of hydrogen for its reabsorption. There were insufficient data to indicate whether the inhibition of sodium reabsorption in subjects with heart failure was significantly more or less than in control subjects. Furthermore, this study provides no data to show whether the severity of edema present in a cardiac patient modifies the effect of 6063 on sodium excretion. The degree of increase in sodium excretion due to carbonic anhydrase inhibition appeared to be significantly influenced by the previous dietary intake of sodium. This relationship existed whether or not congestive heart failure was present. These observations suggest that variations in the dietary intake of sodium influence the quantity of sodium excretion related to urine acidification either by altering sodium concentration in the tubular cell or by some other mechanism. The effect of carbonic anhydrase inhibition on sodium excretion appears to be limited or modified by some such mechanism.

The interpretation of the increase in potassium excretion following 6063 is hampered by uncertainty as to the occurrence and quantity of tubular reabsorption or secretion of this ion in the distal tubule under normal circumstances. Studies of potassium excretion following the administration of 6063 to normal dogs (13) indicated that increased potassium excretion after this drug was due to increased tubular secretion of potassium. Berliner, Kennedy, and Orloff (13) suggested that there was competition between hydrogen and potassium for secretion by the tubular cells; inhibition of hydrogen secretion by the carbonic anhydrase inhibitor, 6063, permitted the increased secretion of potassium. The increased potassium secretion following 6063 in our human subjects is compatible with this concept of hydrogen-potassium competition.

It is apparent that the increased bicarbonate excretion accounted for almost all of the increase in anions excreted. Furthermore, it may be postulated that the inhibition of carbonic anhydrase and the consequent prevention of hydrogen ion secretion have their major effects upon the bicarbonate ion in the tubular urine, for unless it reacts with the hydrogen ion the bicarbonate is not reabsorbed. The sodium concentration in glomerular

filtrate greatly exceeds bicarbonate concentration, while in the urine excreted after 6063 the bicarbonate concentration was relatively higher. If the mechanism involved were simply inhibition of the exchange of hydrogen ion for sodium ion, one would expect the rise in excretion rates for sodium and bicarbonate to approximate each other. In this series of cases the rise in bicarbonate excretion exceeded that of sodium in five cases. This might be explained by a simultaneous inhibition of hydrogen-potassium exchange or, as has been suggested by Berliner and his co-workers (22), an exchange between sodium and potassium may take place under appropriate circumstances, with sodium being reabsorbed and potassium excreted.

We have no evidence to indicate whether the slight increases in chloride and phosphate excretion were direct consequences of the inhibition of carbonic anhydrase activity. It may be that the increase in total electrolyte excretion resulted in an increased rate of flow and this in turn caused a slight increase in the excretion of chloride (23). There are considerable experimental data to indicate that a simple osmotic diuresis will not increase phosphate excretion (23, 24). But studies on a group of patients similar to the subjects presented in this paper and under similar conditions of hydration showed a slight but consistent rise in phosphorus excretion during a urea diuresis (25). Since acidosis is associated with an increased phosphate excretion (26–28), it may be considered as a possible cause of the increment in phosphate excretion after 6063. However, the degree of acidosis following the latter drug was usually slight.

Although ordinarily acidosis greatly increases the ammonia excretion for any given urinary pH, ammonia production following 6063 was greatly diminished despite the presence of acidosis. As has been previously suggested by animal experiments by Ferguson (29) and by ammonium chloride experiments by Pitts (30), the results presented in this paper support the theory that the presence of hydrogen ions in the tubular urine is of considerably more importance in stimulating the excretion of ammonia by the kidney than acidosis of the blood. The extreme reduction in ammonia excretion following 6063 appears to be due to the inhibition of tubular secretion by hydrogen ion and consequent reduction in hydrogen ion concentration in the urine. However, ammonia excretion did not completely cease. This may indicate either that tubular secretion of hydrogen was incompletely inhibited or that the hydrogen ions necessary for ammonia excretion were made available by the buffer acids, carbonic acid or acid phosphate, in the alkaline tubular urine.

It is of interest to note that while both the mercurial and 6063 produced a sodium diuresis, after the former the urine usually contains a relative excess of chloride over sodium (31), whereas after the latter it contains a relative excess of bicarbonate and sodium over chloride. If the toxic effect of the mercurial on the tubule impairs tubular reabsorption of all electrolytes including sodium, chloride and bicarbonate, then most of the bicarbonate and some of the sodium may be reabsorbed subsequently by the acidifying mechanism. The chloride is not reabsorbed in the process of acidification of the urine and, therefore, is excreted in excess of sodium and bicarbonate. After 6063, only the electrolytes believed to be involved in the acidification are excreted in significantly larger amounts. There is only a slight indirect effect upon chloride excretion, a moderate increase in sodium excretion, and a maximal increase in bicarbonate excretion. Consequently there is an excess of bicarbonate excretion with acidosis after 6063 and an excess of chloride over sodium excretion with alkalosis after a mercurial.

SUMMARY AND CONCLUSIONS

1. The specific carbonic anhydrase inhibitor 6063 prevented acidification of the urine by the normal human kidney and the kidneys of patients with heart failure.

2. Bicarbonate, sodium, and potassium excretion were markedly increased whereas chloride and phosphate excretion were increased only slightly and ammonia excretion diminished.

3. The absolute increment in sodium excretion in these studies appeared to be related to the sodium intake in the days prior to the experiments and slightly if at all to the presence or absence of heart failure in these cases.

4. Moderate increases in the excretion of water and moderate weight loss in twenty-four hours occurred despite the fact that the cardiac patients had become free of edema previously or were refractory to mercurial diuretics.

5. There was slight acidosis but no significant change in the plasma concentration of sodium and potassium following a single intravenous injection of 6063. However, an intravenous injection of 6063 following two days of oral 6063 led to blood bicarbonate values in the range of 15 milliequivalents per liter. Despite this acidosis there was a marked excretion of sodium and bicarbonate, and the urine was alkaline.

6. The possible mechanism by which the carbonic anhydrase inhibitor, 6063, produced the observed electrolyte changes was discussed. It was postulated that bicarbonate reabsorption in the distal tubule depended upon hydrogen ion secretion, whereas reabsorption of the corresponding sodium ions could be accomplished by either hydrogen ion or potassium ion secretion.

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INTERRELATIONSHIPS AMONG PULMONARY "CAPILLARY" PRESSURE, BLOOD FLOW AND VALVE SIZE IN MITRAL STENOSIS. THE LIMITED REGULATORY EFFECTS OF THE PULMONARY VASCULAR RESISTANCE [1]

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Evidence from several sources clearly indicates that the pulmonary vascular bed is abnormal in patients with mitral stenosis. Pathologic studies of the lungs have revealed varying degrees of arteriolar sclerosis, thickening of the basement membrane of the alveolar capillaries, pericapillary edema and fibrosis (1–3). Increased resistance to the flow of blood through the lungs has been confirmed by measurements made with the technique of cardiac catheterization (4–6). In a study of lung biopsy sections obtained at the time of mitral commissurotomy a direct relationship between the magnitude of the pulmonary vascular resistance and the degree of vascular sclerosis was observed (3). In addition to the anatomic alterations, physiologic changes, perhaps in the nature of increased vascular tone, have been implicated. The striking decreases in pulmonary vascular resistance observed within four to six weeks following successful commissurotomy of the mitral valve (4, 6) and acutely after the administration of certain vasodilating agents (7) suggest participation of reversible physiologic factors. The pathogenesis of neither the anatomic nor physiologic changes is clearly understood.

Dexter and his co-workers contend that the pulmonary arterioles constrict when the pressure in the capillaries of the lungs attains plasma colloid osmotic levels, as it may in left ventricular failure or in mitral stenosis (8). In studies of mitral stenosis this group of workers have demonstrated an inverse relationship between the size of the calculated mitral valve orifice and the pulmonary arteriolar resistance—the smaller the mitral valve area, the larger the resistance (9). The restriction of pulmonary blood flow by the constricted arterioles has been thought of as protective in nature. Since increases in the rate of flow through a stenotic mitral valve require elevations of left atrial, pulmonary venous, and capillary pressures that frequently exceed plasma protein osmotic pressure it is argued that arteriolar constriction prevents pulmonary edema (8, 9).

In this paper certain data obtained by cardiac catheterization of 36 patients with mitral stenosis are presented. Only those observations relative to the interrelationships of mitral valve area and the vascular resistance, "capillary" pressure, blood flow and the occurrence of edema in the lungs are presented. Our data differ from those of Dexter and his co-workers (8, 9) in that they failed to demonstrate: *1*) evidence of pulmonary arteriolar constriction occurring as a response to elevation of the pulmonary "capillary" pressure; *2*) any reliable correlation between pulmonary vascular resistance and mitral valve orifice area; *3*) that an increased pulmonary vascular resistance prevents increases in pulmonary "capillary" pressure high enough to cause pulmonary edema. More comprehensive data on the pulmonary circulation in mitral stenosis have been published previously (4).

SUBJECTS AND METHODS

Subjects

Thirty-six hospitalized patients with mitral stenosis of varying severity were used in this study. The majority were catheterized as part of pre-mitral commissurotomy evaluation and were moderately to severely restricted by their heart disease. All were considered to be compensated at the time of study; those with auricular fibrillation

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and a number of those with normal rhythm were maintained on digitalis.

All but a few subjects had apical systolic murmurs, but clinically and at operation the predominant lesion was mitral stenosis. In 16 (44 per cent) evidence of tricuspid insufficiency was noted in the right atrial pressure tracing obtained at cardiac catheterization. In none was tricuspid stenosis apparent. The high incidence of clinically inapparent tricuspid insufficiency in severe mitral stenosis has been noted previously (4).

Each subject was studied in the resting state, five hours post-absorptive. Sodium amytal 0.2 gm. was administered one-half hour prior to the procedure.

Methods

The technique of cardiac catheterization and the analytic methods were reported previously (4). Pulmonary "capillary," pulmonary arterial, right ventricular pressures and cardiac output were determined at rest. These observations were repeated during the last two minutes of a five-minute exercise, which consisted of flexion and extension of the legs in a bicycling manner at the rate of one cycle per two seconds.

The area of the mitral valve orifice (MVA) was calculated by the method of Gorlin and Gorlin (10) except that the diastolic filling period, during which blood flows across the mitral valve, was determined from the right ventricular or pulmonary arterial pressure tracing. In 21 patients where sufficient data were available the MVA was calculated during both rest and exercise (Table I). The average difference between the values from the two calculations was 0.12 sq. cm.; the range of difference was 0.01 to 0.34 sq. cm. The close agreement between the two sets of calculated valve areas affords an internal check on the reliability of the methods of obtaining the data and the calculations. This, however, does not validate the Gorlin formula since it may yield considerably inaccurate values in the presence of marked mitral insufficiency. Experience with this and other groups of patients with *small* degrees of mitral insufficiency has indicated that the MVA usually agrees closely with the area determined at autopsy or commissurotomy. The Gorlin formula has been used here since it is the only available method of estimating MVA in the living patient and because it has been used extensively by other workers. The calculated MVA was related to body surface area (BSA) and expressed as MVA index. The pulmonary capillary pressure is regarded by many (10–13) as a reasonable estimate of the pressure in the pulmonary veins and left atrium and is used interchangeably with the latter in the discussion. The true pressure in the capillaries of the lungs is thought to be a few mm. Hg higher than the "PC" pressure (12).

TABLE I

Mitral valve orifice area in sq. cm. calculated from rest and exercise data

Case No.	Rest	Exercise	Difference
1	2.68	2.71	.03
5	1.53	1.54	.01
7	1.31	1.24	.07
9	1.32	1.15	.17
11	1.29	1.62	.33
12	1.27	0.93	.34
13	1.19	1.15	.04
14	1.21	1.17	.04
16	1.27	1.08	.19
17	0.92	0.73	.19
19	1.00	0.94	.06
20	1.10	1.34	.24
22	0.98	1.00	.02
23	1.21	1.39	.18
26	1.04	0.95	.09
29	0.73	0.61	.12
30	0.73	0.81	.08
31	0.64	0.72	.08
32	0.70	0.81	.11
33	0.64	0.67	.03
34	0.51	0.45	.06
Average	1.11	1.10	0.12

The pulmonary vascular resistance (PVR) was determined by the formula of Apéria (14):

$$PVR = \frac{(PAm - \text{"PC"m}) \times 1.332 \times 60}{CO}$$

where

PVR = pulmonary vascular resistance in dynes-sec.-cm.$^{-5}$
PAm = mean pulmonary arterial pressure in mm. Hg
"PC"m = mean pulmonary "capillary" pressure in mm. Hg
CO = cardiac output in L./min.

This measurement includes the resistance of the pulmonary capillaries since the pressure decrement from pulmonary artery to vein is used.

RESULTS

"PC" pressure at rest and exercise

In two subjects, resting "PC" pressure was almost normal; the MVA index was greater than 1.4 sq. cm. in both. In the remainder of the subjects MVA indices were below 1.4 sq. cm. and there was an increase in the resting "PC" pressure to a strikingly similar level (Table II). The mean value was 28 mm. Hg with a narrow standard deviation of ± 3.2 mm. Despite variations in the PVR the hydrostatic pressure in the pulmonary capillaries remained in the range of plasma colloid osmotic pressure (*i.e.*, 25 to 30 mm. Hg) and significant pulmonary edema was not present in any of the subjects.

The adjustment in "PC" pressure was adequate for the maintenance of a normal cardiac index at rest when the MVA index was greater than 0.9 sq. cm. As the mitral valve narrowed below this figure the cardiac index declined directly ($r = 0.897 \pm 0.037$) (Figure 1). To facilitate *normal*

TABLE II

Physical characteristics, mitral valve area indices, cardiac indices, pulmonary "capillary" pressures and vascular resistances during rest and exercise in mitral stenosis

Case No.	Age and sex	Body surface area sq. m.	Mitral valve area index sq. cm./ sq. m. bsa	Cardiac index L./min./sq. m. bsa		Pulmonary "capillary" pressure mm. Hg		Pulmonary vascular resistance dynes-sec.—cm^{-5}	
				Rest	Exercise	Rest	Exercise	Rest	Exercise
Normal			2.3–3.3	3.4	4.4+	8–10	12	100	<100
1	26M	1.75	1.53	3.53	4.88	13	26	91	112
2	18F	1.74	1.44	3.09	5.83	11	28	59	47
3	36F	1.57	1.38	3.57	3.68	29	40	242	263
4	24M	1.80	1.01	3.03	4.86	28	46	249	347
5	36F	1.54	0.99	3.31	4.21	21	35	110	62
6	22M	1.66	—	3.44	4.28	33	—	182	—
7	44F	1.32	0.99	2.92	3.07	29	45	457	572
8	37F	1.54	0.88	3.18	4.16	29	45	212	287
9	21F	1.58	0.84	3.40	3.72	25	44	179	177
10	29F	1.44	0.82	2.58	2.32	30	40	172	311
11	41M	1.60	0.81	2.83	3.79	32	54	194	434
12	24F	1.56	0.81	2.26	2.15	29	40	772	1237
13	22F	1.57	0.76	2.36	3.87	29	45	216	302
14	31F	1.60	0.76	1.91	1.96	29	37	1201	1200
15	29F	1.84	0.74	2.31	2.52	29	44	132	103
16	41M	1.73	0.73	2.22	2.28	24	40	271	324
17	34F	1.33	0.69	1.47	2.03	25	35	2049	1954
18	38F	1.49	0.67	2.28	2.66	23	33	165	161
19	49F	1.50	0.67	2.17	2.96	25	40	885	612
20	37F	1.67	0.66	2.02	2.70	30	40	474	744
21	24F	1.48	0.64	1.80	—	28	—	2065	—
22	30F	1.57	0.62	3.08	3.16	32	45	347	500
23	30M	1.99	0.61	2.66	4.08	23	47	76	89
24	45F	1.48	0.61	2.31	2.20	28	—	421	—
25	35M	1.55	0.60	2.39	2.71	28	57	216	514
26	37F	1.72	0.60	1.86	2.98	26	45	200	265
27	40M	1.83	0.58	2.47	3.54	29	—	318	—
28	25M	1.88	0.55	2.22	—	33	54	594	—
29	46F	1.50	0.49	2.03	2.29	29	55	394	326
30	43F	1.51	0.48	1.95	2.03	30	35	1223	1354
31	22F	1.35	0.47	2.39	2.85	27	42	223	208
32	45F	1.53	0.46	2.47	2.44	26	34	190	429
33	32F	1.47	0.43	2.00	2.52	28	35	326	324
34	43F	1.25	0.41	1.87	1.80	32	52	1127	1172
35	39M	1.69	0.40	1.82	2.80	31	—	417	—
36	37F	1.58	0.37	1.87	2.20	36	—	135	—
Average		1.59	0.73	2.47	3.10	27	42	461	498

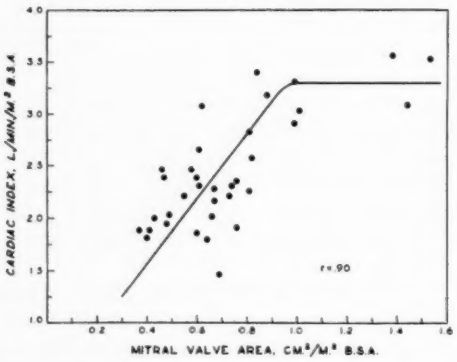

FIG. 1. RELATIONSHIP OF CARDIAC INDEX TO MITRAL VALVE AREA INDEX IN MITRAL STENOSIS

blood flow across such small mitral orifices, "PC" pressures larger than those encountered would have been required.

During exercise "PC" pressure rose abruptly to levels well above plasma protein osmotic pressure. The degree of increase above the resting value did not correlate with the PVR ($r = -0.310 \pm 0.168$). Many subjects with greatly increased PVR had marked elevation of "PC" pressure with exercise. It was apparent that increase in cardiac output was not solely responsible for this change since only 15 subjects had adequate increases in output of one L./min./sq. M. BSA or more. In the remainder, cardiac index increased slightly, not at all, or even decreased. Marked tachycardia was frequent during exercise. Since

the diastolic period of the cardiac cycle is shortened with tachycardia, "PC" pressure also rose in association with restriction of the time allotted in the cardiac cycle for the flow of blood across the stenotic mitral valve (15).

Although "PC" pressure was well elevated above plasma colloid osmotic pressure during the five minute exercise period, acute pulmonary edema occurred in only one subject (subject 16). This patient developed marked orthopnea, cough and many moist rales in his lungs during the fourth minute of exercise. His "PC" pressure was 54 mm. Hg and PVR 594 dynes-sec.-cm.$^{-5}$, a value six times normal. The occasional appearance of a few rales at the lung bases was the only sign of pulmonary edema in the other subjects.

Pulmonary vascular resistance (PVR)

The PVR varied from normal to 20 times normal (Table II). Correlation between PVR and mitral valve size was poor in both a direct and logarithmic comparison ($r = -0.316 \pm 0.154$ and $r = -0.435 \pm 0.139$, respectively). A large PVR, however, was most frequently but not consistently encountered among patients with very small mitral valve orifices. As example: PVR among subjects with MVA indices of 0.4 to 0.5 varied from normal to 1,223 dynes-sec.-cm.$^{-5}$.

There was an inverse logarithmic correlation between the cardiac output and PVR during both rest ($r = -0.707 \pm 0.085$) and exercise ($r = -0.757 \pm 0.082$) (Figure 2). However, the degree of change in cardiac output with exercise from resting levels did not correlate well with the PVR ($r = -0.487 \pm 0.133$). In general an increase of PVR was associated with depression of blood flow below that required by the metabolic needs of the body but not necessarily with inability to increase cardiac output with exercise.

DISCUSSION

Depending upon the size of the valve orifice mitral stenosis presents varying degrees of obstruction to the flow of blood from left atrium to left ventricle. The degree of obstruction depends also on the usual blood flow requirements of the individual and therefore on his size. It is apparent that a valve orifice of 1 sq. cm. is physiologically more tolerable in a small child with a body surface area of 1 sq. M. than it would be in a large well-developed male with a 2 sq. M. body surface area.[3] In this report, therefore, in order

[3] A child with a 1 sq. M. body surface area has a resting cardiac output in the range of 3.4 L./min.; a man with a 2 sq. M. body surface area has an output of 6.8 L./min. The effect of a mitral stenosis with an orifice area of 1.0 sq. cm. on the "PC" pressure of each can be calculated from the Gorlin formula:

$$MVA = \frac{\frac{CO}{DFP}}{31\sqrt{\text{"PC"} - 5}}$$

If a diastolic filling period of 30 sec. is assumed the "PC"

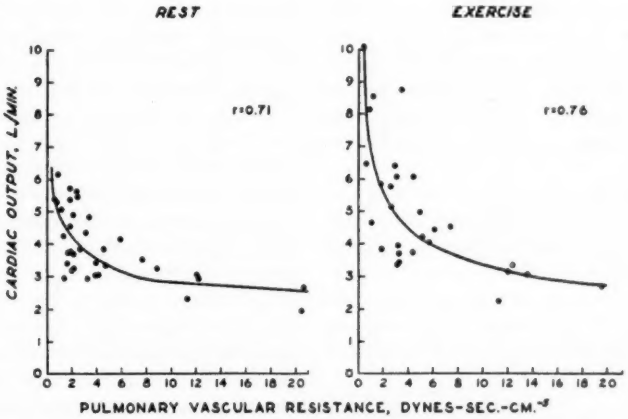

FIG. 2. RELATIONSHIP OF CARDIAC OUTPUT TO PULMONARY VASCULAR RESISTANCE DURING REST AND EXERCISE IN MITRAL STENOSIS

to compare the effects of stenosis among subjects of varying size the MVA was referred to the body surface area and expressed as MVA index in the same way as is conventional for the cardiac output.

With MVA indices greater than 1.4 sq. cm. only modest increases of left atrial, pulmonary venous, capillary and arterial pressures are required to maintain a normal cardiac index at rest. With smaller mitral orifices the maintenance of cardiac output is accomplished at the expense of elevation of pulmonary venous pressure to plasma colloid osmotic levels (*i.e.* 25 to 30 mm. Hg). Since the resting pulmonary venous pressure remains selectively fixed at 28 ± 3.2 mm. Hg, progressive narrowing of the MVA index below 0.9 sq. cm. is associated with progressive and marked diminution in cardiac output.

It appears therefore that in severe mitral stenosis selective fixation of the resting pulmonary venous pressure in the plasma protein osmotic range is effected by reduction of cardiac output. The mechanism by which cardiac output is reduced is not known. Dexter (8), Lewis (9) and their associates believe that the increased flow resistance of the pulmonary arterioles is responsible. In the present study an inverse correlation between cardiac output and PVR was observed. It is not likely, however, that lowering of blood flow is accomplished by an increased PVR since output was similarly depressed among patients with identical MVA regardless of the degree of PVR change. Nor can regulation of cardiac output be ascribed to inability of the right ventricle to pump blood since many of these patients were able to increase their output with exercise. It is possible that the cardiac output is regulated by reflexes originating from pressure receptors in the left atrium or the pulmonary veins.

Other investigators (5, 6) have not observed as close an agreement in resting "PC" pressure among subjects with mitral stenosis as reported here. It is possible that the state of cardiac compensation was more variable in their patients. This is true in the case of one report in which several patients with failure were included (6). None of the present group of subjects was decompensated at the time of cardiac catheterization.

It is of interest that the direct measurements of left atrial pressure in 10 patients with severe mitral stenosis reported by Bland (16) averaged 32 mm. Hg (range: 27 to 39 mm.), a value only slightly higher than the average "PC" pressure in our patients. These measurements, however, were made in the open chest at time of operation and are not accompanied by a zero reference level.

Experimentally edema of the lungs occurs when the pulmonary venous pressure attains plasma colloid osmotic levels (17, 18). In the present study the absence of significant pulmonary edema with pulmonary venous pressures in such a range at rest, and well above during brief exercise, is a matter of considerable interest. These facts suggest that the alveolar-capillary structures in mitral stenosis are not normally permeable to fluid. The anatomic alterations (*i.e.* capillary basement membrane thickening and pericapillary fibrosis) that very commonly exist in these structures probably impede transudation of fluid from the pulmonary capillaries out into the alveoli. This is suggested by the frequency with which interstitial pulmonary edema without edema fluid in the alveolar spaces is observed in lung biopsy sections from patients with mitral stenosis (3).

The data of Gorlin and his co-workers (5) provide additional evidence of decreased permeability of the alveolar membranes to fluid. In their patients with mitral stenosis pulmonary edema at rest was noted only in those with pulmonary "capillary" pressures averaging 40 mm. Hg (a value considerably in excess of plasma protein osmotic pressure): whereas those with pulmonary "capillary" pressures in the range of osmotic pressure (average 24 mm. Hg) did not manifest pulmonary edema at rest. From these observations it appears that regulation of the resting pulmonary venous pressure at the plasma protein osmotic level and decreased alveolar membrane permeability to fluid combine to prevent the development of pulmonary edema in the patient with severe mitral stenosis. Clinically detectable pulmonary edema is produced only by prolonged elevations of pulmonary venous pressure well in excess of plasma protein osmotic pressure.

This study sheds no light on the pathogenesis of the increased resistance to flow of the pulmonary

pressure in the child at rest would be 18 mm. Hg and in the man 58 mm. Hg. The effect of a stenotic mitral orifice of similar size on the pulmonary circuit pressures in these different-sized individuals is obviously marked.

vessels. It is evident that very small mitral orifices predispose to the development of pulmonary vascular changes but are not always associated with them. In view of the frequently normal and wide range of values for PVR in association with "PC" pressures of 25 to 30 mm. Hg, there is little evidence to indicate that arteriolar contriction occurs as a response to elevations of pulmonary venous pressure to the plasma protein osmotic range, as has been suggested previously (8, 9). The close relationship between measured PVR and degree of vascular sclerosis observed in lung biopsy sections (3), suggests that these structural changes in the vessels are primarily responsible for the resistance to flow.

Although there is some tendency of a large resistance to damp increases of pulmonary blood flow during exercise, large changes in pulmonary venous pressure are not prevented. Indeed the degree of increase of both blood flow and pulmonary venous pressure with exercise cannot be related reliably to the state of the PVR. In the light of these observations the concept of an increased PVR performing an active protective function in mitral stenosis must be held in doubt.

It has been pointed out repeatedly that pulmonary vascular changes cause a disproportionate rise of arterial pressure with respect to the venous pressure in the lungs (4–6). Right ventricular work is consequently increased. In some instances more work is expended by the right ventricle in driving blood through the pulmonary vessels than through the stenotic mitral valve (4). Large increments in PVR thereby accelerate the process of right ventricular hypertrophy, dilatation and failure.

SUMMARY

The maintenance of blood flow across the stenotic mitral valve is accompanied by an increase in the left atrial and pulmonary venous pressure. In a group of patients with severe mitral stenosis but not in cardiac failure pulmonary "capillary" pressure at rest was found to be fixed within the range of the plasma protein osmotic pressure. Fixation of the pulmonary "capillary" pressure was accomplished at the expense of the resting cardiac output which varied directly with the size of the mitral orifice. The mechanism by which reduction of cardiac output (needed for regulation of pulmonary "capillary" pressure) was accomplished is not known. It was not achieved by an increased pulmonary vascular resistance since there was no correlation between mitral valve size and pulmonary vascular change. The latter varied widely among individuals with similar valve areas.

Large increases in pulmonary "capillary" pressure occurred during exercise and were not prevented by an increased pulmonary vascular resistance. There was no evidence that pulmonary arteriolar constriction occurs when the pulmonary "capillary" pressure attains plasma protein osmotic levels. Despite pulmonary "capillary" pressures at and above such levels detectable alveolar edema was rare. This suggests that changes in the alveolar membrane rather than in the pulmonary arterial tree protect against pulmonary edema. The increased pulmonary vascular resistance of mitral stenosis is primarily the result of anatomic changes in the vessels.

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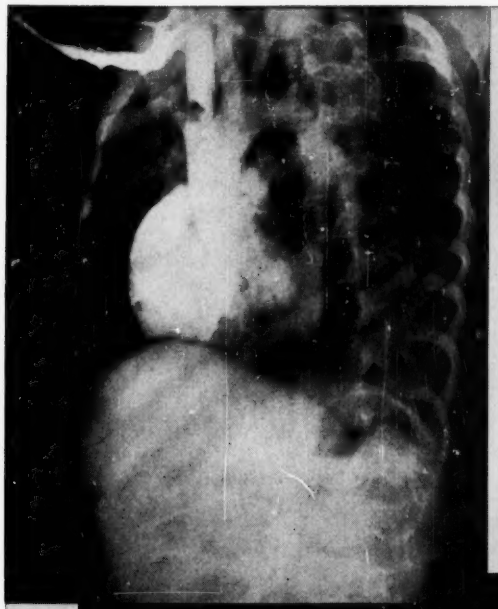

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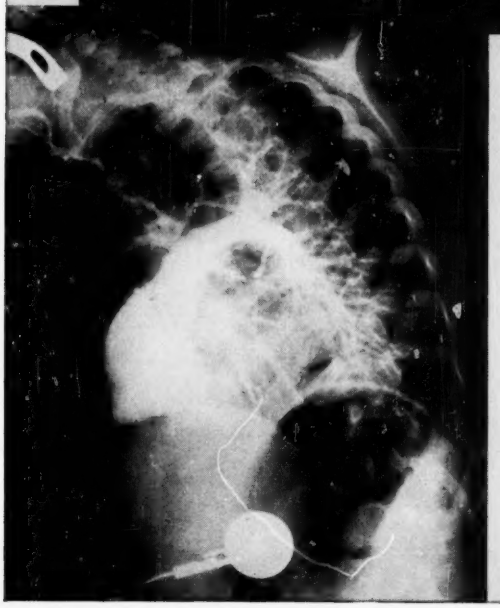

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